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(54) Title: METHOD OF PREVENTING THE INJURY OR DEATH OF RETINAL CELLS AND TREATING OCULAR DIS-
EASES

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(57) Abstract: The present invention relates to the use of PRO polypeptides to delay, prevent or rescue retinal neurons, includ-
ing photoreceptors, other retinal cells or supportive cells (e.g. Müller cells or RPE cells) from injury and/or death. Conditions
comprehended by treatment of the present PRO polypeptides (including variants), antibodies, compositions and articles of manu-
facture include: retinal detachment, age-related and other maculopathies, photic retinopathies, surgery-induced retinopathies (either
mechanically or light-induced), toxic retinopathies including those resulting from foreign bodies in the eye, diabetic retinopathies,
retinopathy of prematurity, viral retinopathies such as CMV or HIV retinopathy related to AIDS, uveitis, ischemic retinopathies due
to venous or arterial occlusion or other vascular disorder, retinopathies due to trauma or penetrating lesions of the eye, peripheral
vitreoretinopathy, and inherited retinal degenerations. Exemplary retinal degenerations include e.g., hereditary spastic paraplegia
with retinal degeneration (Kjellin and Barnard-Scholz syndromes), retinitis pigmentosa, Stargardt disease, Usher syndrome (retinitis
pigmentosa with congenital hearing loss), and Refsum syndrome (retinitis pigmentosa, hereditary hearing loss, and polyneuropathy).
Additional disorders which result in death of retinal neurons include, retinal tears, detachment of the retina and pigment epithelium,
degenerative myopia, acute retinal necrosis syndrome (ARN), traumatic chorioretinopathies or contusion (Purtscher's Retinopathy)
and edema.



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METHOD OF PREVENTING THE INJURY OR DEATH OF RETINAL CELLS AND TREATING OCULAR DISEASES

BACKGROUND

5

The present invention relates to a method of promoting retinal cell survival as well as preventing retinal cell degradation and to the treatment of diseases or conditions which are characterized by retinal neuron death.

10 The retina is the light-sensitive portion of the eye. The retina contains the cones and rods (collectively "photoreceptors"), the photosensitive cells. The rods contain rhodopsin, the rod photopigment, and the cones contain 3 distinct photopigments, which respond to light and transmit signals through successive neurons to ultimately trigger a neural discharge in the output cells of the retina, the ganglion cells. The signal is carried by the optic nerve to the visual cortex where it is registered as a visual stimulus.

15 In the center of the retina is the macula lutea, which is about 1/3 to 1/2 cm in diameter. The macula provides detailed vision, particularly in the center (the fovea), because the cones are higher in density. Blood vessels, ganglion cells, inner nuclear layer and cells, and the plexiform layers are all displaced to one side (rather than resting above the ones), thereby allowing light a more direct path to the cones.

20 Under the retina is the choroid, a collection of blood vessels embedded within a fibrous tissue, and the pigmented epithelium (PE), which overlays the choroid layer. The choroidal blood vessels provide nutrition to the retina (particularly its visual cells). The choroid and PE are found at the posterior of the eye.

25 The retinal pigment epithelial (RPE) cells, which make up the PE, produce, store and transport a variety of factors that are responsible for the normal function and survival of photoreceptors. RPE are multifunctional cells that transport metabolites to the photoreceptors from their blood supply, the chorio capillaris of the eye. The RPE cells also function to recycle vitamin A as it moves between the photoreceptors and the RPE during light and dark adaptation. RPE cells also function as macrophages, phagocytizing the rhythmically-shed tips of the outer segments of rods and cones, which are produced in the normal course of cell physiology. Various ions, proteins and water move between the RPE cells and the interphotoreceptor space, and these molecules ultimately effect the metabolism and viability of the photoreceptors.

30 The Müller cell is the most prominent glial cell within the retina, and could also be important for maintaining the viability of visual cells. Müller cells traverse the entire retina in a radial direction from the ganglion cells to the external limiting membrane, a photoreceptor-photoreceptor and Müller cell-photoreceptor contact point. In addition to providing structural support, Müller cells regulate the control of ionic concentrations, degradation of neurotransmitter, removal of certain metabolites and may be a source of important factors that promote the normal differentiation of photoreceptor cells. Kljavin and Reh (1991), *J. Neuroscience* 11: 2985-2994. Although a search for defects in Müller cells has not specifically been examined, any disease or injury affecting their normal function most likely would have a dramatic influence

on the health of rods and cones. Finally, the death of rod photoreceptors may influence the viability of cones. One common feature in degenerations involving mutations in rod specific genes (*i.e.*, rhodopsin) is that cones also eventually die. The reason for the loss of cones has not been determined, although it has been suggested that dying rods may release endotoxins. Bird (1992), *Opthal. Pediatric. Genet.* 13: 57-66.

5 Diseases or injury to the retina can lead to blindness if retinal cells are injured or killed. The photoreceptor cells are particularly susceptible to injury since they are often the first cells to degenerate or suffer damage as a result of a traumatizing event or condition. Hereditary defects in specific photoreceptor genes, retinal detachment, circulatory disorders, overexposure to light, toxic effects to drugs and nutritional deficiencies are among the wide array of causes that can result in the death of photoreceptor cells.

10 Developmental and hereditary diseases of the retina account for around 20 percent of all legal blindness in the United States. *Report of the Retinal and Choroidal Panel: Vision Research - A National Plan 1983-1987*, vol. 2, part 1, summary page 2. For example, *retinitis pigmentosa* (RP), a genetic based progressive disease is characterized by incremental loss of peripheral vision and night blindness, which is due in large part to the loss of photoreceptor cells. RP is a group of hereditary diseases and presently afflicts about one

15 in 3000 persons worldwide. Wong, F. (1995) *Arch. Ophthalmol.* 113: 1245-47. Total blindness is the usual outcome in more progressive stages of this disease. Macular degeneration, another major cause of blindness, is a complex group of disorders that affects the central or predominantly cone portion of the retina. Cones are primarily responsible for acute vision. Diabetic retinopathy, a frequent complication in individuals with diabetes mellitus, is estimated to be the fifth leading cause of new blindness. However, it is

20 the second leading cause of blindness among individuals of 45-74 years of age. Moreover, these problems are only expected to get worse as the general population ages.

Photoreceptor degeneration may also occur as a result of overexposure to light, various environmental trauma or by any pathological condition characterized by death or injury of retinal neurons or photoreceptors.

25 Photoreceptor loss may also be influenced by cellular or extracellular retinal components. The primary example of extracellular stimulus is related to the close association between the pigment epithelium (PE) and the photoreceptor cells. As mentioned previously, the PE transports metabolites to and from the photoreceptors as well as removes discarded cellular material. Retinal detachment, which involves the separation of the neural retina from the PE leads to photoreceptor death. Furthermore, the degree of cell loss

30 is dependent upon the duration of the separation. Gouras *et al.*, *IOVS* 32: 3167-3174 (1991).

Additionally, diseases of the PE can lead to photoreceptor cell loss. The primary example of this is the Royal College of Surgeons (RCS) rat, which has an inherited retinal dystrophy due to a defect in the PE, resulting in photoreceptor cell death during the normal course of the animal's life. Mullen & LaVail, *Science* 192: 799-801 (1976). In this animal, the PE are unable to phagocytize outersegment debris which

35 accumulates between the photoreceptor cells and the PE, and as a result, provide a useful model system to study the role of trophic factors on the retina. A delay of photoreceptor death is obtained through the proximal placement of normal PE cells both in experimental chimeras, Mullen & LaVail, *supra* and by transplantation of PE from healthy animals. Li & Turner, *Exp. Eye Res.* 47: 911-917 (1988); Sheedlo *et al.*,

Int. Rev. Cytol. 138: 1-49 (1992); Lavail *et al.*, *Exp. Eye Res.* 55: 555-562 (1992); Lavail *et al.*, *PNAS* 89: 11249-11253 (1992). In all of these experiments, the "rescue" extended beyond the boundaries of the normal PE cells, and suggests the presence of diffusible trophic factor(s) produced by the PE cells.

Another useful animal model is the albino rat. In this animal, normal illumination levels of light, if continuous, can cause complete degeneration of photoreceptors. Results obtained using such rats as a model to identify survival enhancing factors appear to correlate well with data obtained using RCS rats. Moreover, different factors can be compared and complications can be assessed more quickly in the light damage model than can be assessed by testing factors in models which are based on the slowly evolving dystrophy of the RCS rat.

Using albino rats, it has been determined that a number of agents, when administered systemically (intraperitoneally) can be used to ameliorate retinal cell death or injury caused by exposure to light. In general, exposure to light generates oxygen free radicals and lipid peroxidation products. Accordingly, compounds that act as antioxidants or as scavengers of oxygen free radicals reduce photoreceptor degeneration. Agents such as ascorbate, Organisciak *et al.*, *Invest. Ophthalm. & Vis. Sci.* 26: 1580-1588 (1985), flunarizine, Edward *et al.*, *Arch. Ophthalmol.* 109: 554-562 (1991), and dimethylthiourea, Lam *et al.*, *Arch. Ophthalmol.* 108: 1751-1757 (1990) have been used to ameliorate the damaging effects of constant light. There is no evidence, however, that these compounds will act to ameliorate other forms of photoreceptor degeneration and their administration can potentiate harmful side effects. Further, these studies are limited because they utilize systemic delivery, which does not provide an adequate means of assessing the effectiveness of a particular factor. As a result, it is nearly impossible to assess the amount of agent that actually reaches the retina. A large amount of agent must be injected to attain a sufficient concentration at the site of the retina. In addition, systemic toxic effects may result from the injection of certain agents.

Traditional approaches to treating the loss of vision due to photoreceptor cell death has taken at least two routes: (1) replacing the defective cells by physical transplantation; and (2) slowing, arresting or preventing the process of degeneration. The transplantation of healthy pigment epithelium cells into a degenerating retina or one which has defective epithelium cells can rescue photoreceptor cells from dying. Sheedlo *et al.*, *Int. Rev. Cytol.* 138: 1-49 (1992); Lavail *et al.*, *Exp. Eye Res.* 55: 555-562 (1992); and Lavail *et al.*, *PNAS* 89: 11249-11253 (1992) PE transplants in humans have been attempted, but the results have been less than satisfactory. Peyman *et al.*, *Ophthalm. Surg.* 22: 102-108 (1991). More promising, but as yet unproven is the transfer of embryonic retina containing mostly undifferentiated progenitor cells, which can differentiate in response to environmental cues into appropriate missing cell types. Cepko, *Ann. Rev. Neurosci.* 12: 47-65 (1989). In conclusion, therapy via functional integration of transplanted retinal cells into a human host retinas remain unproven.

Other strategies have focused on "rescuing" or slowing the loss of visual cells. These techniques include corrective gene therapy, limiting the exposure to normal light during disease, vitamin A supplemented diets and the administration of growth factors to damaged or degenerating eyes. However, these treatment schemes have several limitations. For example, gene therapy or the insertion of a

replacement allele into the cells carrying the known mutation may prove problematic. Milam, *Curr. Opin. Neurobiology* 3: 797-804 (1993). Since rods and cones are somewhat inaccessible, it might be difficult to deliver replacement genes to them. Moreover, the use of retroviral vectors for insertion of replacement genes is limited to dividing cells, such as cultured PE, whereas post-mitotic neurons, e.g. photoreceptors, will require other viral vectors such as HSV (Herpes simplex virus) for effective delivery. Finally, gene replacement may not correct a disease where the mutant gene product is deleterious to the cell, but may be more useful for correcting defects due to the loss-of-function of a gene product, as is found in most recessive disorders.

Limiting light exposure, a low technology conventional approach to attenuating vision loss, typically using such approaches as eye-patches, dark goggles, etc. is impractical, since the practical effect of the treatment is the same as the disease itself: blindness and inability to detect light.

Vitamin A has been observed to halt the decline of retinal function by over 20% as administered over the course of 4-6 years in the progression of patients with *retinitis pigmentosa* (RP). Berson, E.L. *et al.*, *Arch Ophthalmol.* 111: 761-772 (1993). While this study did indicate a potential lengthening of years of useful vision, several criticisms of vitamin A therapy exist: (1) the mechanism by which vitamin A (and even vitamin E) alter the progression of RP is unknown; (2) it is not known whether or not patients with different genetic forms of RP will respond to vitamin A therapy; (3) it is not apparent whether or not quantifiable measurements of visual function (*i.e.*, perimetry and visual acuity) revealed any significant benefit from vitamin A therapy; and (4) long term ingestion of vitamin A may have detrimental side effects in other organ systems.

A number of agents, when administered systemically (intraperitoneally) can be used to ameliorate retinal cell death or injury caused by exposure to light. In general, exposure to light generates oxygen free radicals and lipid peroxidation products. It has been suggested that genetically defective photoreceptors are abnormally sensitive to photooxidation from light levels as encountered normally in the environment. Hargrave, P.A. & O'Brien, P.J., *Retinal Degenerations*, Anderson RE *et al.* eds., Boca Raton, FL, CRC Press, p. 517-528 (1991). Compounds that act as antioxidants or as scavengers of oxygen free radicals reduce photoreceptor degeneration. Anti-oxidants or calcium overload blockers (*e.g.* flunarizine) have been reported to prevent degeneration of normal photoreceptors after exposure to high light levels. Rosner *et al.*, *Arch. Ophthalmol* 110: 857-861 (1992); Li *et al. Exp. Eye Res.* 56: 71-78 (1993). Additional success in reducing photoreceptor degeneration has been observed through administration of ascorbate (Organisciak *et al.*, *Invest. Ophthalm. & Vis. Sci.* 26: 1580-1588 (1985)), flunarizine (Edward *et al.*, *Arch. Ophthalmol.* 109: 554-562 (1991)), and dimethylthiourea (Lam *et al.*, *Arch. Ophthalmol.* 108: 1751-1757 (1990)). However, there is no evidence that administration of these compounds will reduce photoreceptor degeneration induced by other than intense light exposure. Moreover, there is great concern that their administration can generate potentially harmful side effects. As a result, the search continues for factors which can somehow protect photoreceptors or even promote their regeneration after light-induced damage.

A particular area of interest is the administration of growth factors. Growth factors have been found to participate in diverse roles such as neuronal differentiation, transmitter specificity, regulation of

programmed cell death, and neurite growth in several regions of the central nervous system. However, only recently has their role been studied during retinal development and disease. An early study indicating that diffusible growth factors can rescue photoreceptor cells from dying was based on a chimeric rat constructed to contain both normal and RCS pigment epithelial cells. The animals were produced by fusing blastula
5 from both normal and RCS rat embryos. Mullen and LaVail, *supra*. In the retina of these chimeras, photoreceptor cells adjacent to RCS PE showed degeneration, and those that were lying next to normal PE were healthy. However, photoreceptor cells that were lying just beyond the immediate contact site of normal PE also appeared healthy, suggesting that photoreceptor-PE contact was not needed, and that normal PE were secreting a putative survival promoting factor.

10 Among the best characterized growth factors in the retina are the acidic and basic fibroblast growth factors (aFGF and bFGF). FGF can be detected through immunohistochemical, biochemical or molecular approaches on a variety of retinal cells including PE, photoreceptor cells and the interphotoreceptor cell matrix (IPM), and a collection of extracellular matrix molecules surrounding photoreceptor cells. Jacquemin
15 *et al.*, *Neurosci. Lett.* 116: 23-28 (1990); Caruelle *et al.*, *J. Cell Biol.* 39: 117-128 (1989); Hageman *et al.*, *PNAS* 88: 6706-6710 (1991); Connolly *et al.*, *IOVS* 32 (suppl.): 754 (1991). Intravitreal injection of basic fibroblast growth factor (bFGF) in the RCS rat or rats with light damaged retina prevents photoreceptor cell degeneration for several month, even as outersegment debris accumulates. Faktorovich *et al.*, *Nature* 347:
20 83-86 (1990). Similar results have been seen when bFGF is injected into the subretinal space, the area between the photoreceptors and the PE. However, even sham operations, or injections of phosphate buffered saline (PBS) in both the RCS rat and light damaged retina can delay photoreceptor cell death. However, the rescue effect is small and localized to the needle track, and differs quantitatively from the effect obtained from bFGF. Faktorovich *et al.*, *supra*; Silverman and Hughes, *Curr. Eye Res.* 9: 183-191
(1990); Sheedlo H.J. *et al.*, *Int. Rev. Cyto.* 138: 1-49 (1992). In these experiments it is likely that various growth factors derived from damaged retinal tissues or macrophages present in the damaged area were
25 locally released. Sheedlo *et al.*, *supra*; Silverman and Hughes, *supra*. Macrophages themselves are known to produce many different growth factors or cytokines, some of which could have photoreceptor survival activity. Rappolee and Werb, *Curr. Top. Microbiol. Immunol.* 181: 87-140 (1992).

Various agents disclosed to have survival-enhancing and/or growth activity on retinal neurons are described in certain issued patents and pending patent applications. These include Transforming Growth
30 Factor- β (TGF- β) (WO 94/01124), brain derived neurotrophic factors (BDNF) (U.S.P. 5,180,820) (U.S.P. 5,438,121) and (WO 91/03568), neurotrophin-4 (NT-4) (WO 93/25684), and insulin-like growth factors (IGF) (WO 93/08826).

Other experiments have shown that intravitreal injections of human subretinal fluid as well as other growth factors can rescue dying photoreceptor cells. For example, one recent study tested eight different
35 factors injected into the retina of rats exposed to constant high intensity light, all showing the ability to delay the degeneration of photoreceptor cells. These include FGF (both acidic and basic forms), brain derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), and interleukin 1 (IL-1). Neurotrophin 3 (NT 3), insulin-like growth factor II (IGF-II), Transforming Growth Factor beta (TGF- β) and the tumor

necrosis factors alpha and beta (TNF- α , TNF- β) also showed survival activity, but to a much lesser degree than the other factors. NGF has been reported to reduce the incidence of apoptosis in diabetic rats in addition to minimizing pericyte loss and acellular occluded capillaries, conditions associated with diabetic retinopathy. Hammes, HP *et al.*, *Molecular Med.* 1(5): 527-534 (1995). However, while it does appear that growth factors can enhance survival of photoreceptors, some of these factors may promote detrimental side effects. For example, injections of bFGF results in an increased incidence of macrophages and cataracts. In addition, bFGF is mitogenic for PE, Müller cells and retinal vascular cells. Faktorovich *et al.*, *supra.*; La Vail *et al.*, *supra.* As a result, suitable growth factors which will not only promote the survival of retinal cells, but lack undesired side effects have yet to be discovered.

SUMMARY

A. Embodiments

The present invention relates to a method of delaying, preventing or rescuing photoreceptor cells from injury or death in a mammal comprising administering a therapeutically effective amount of a PRO polypeptide to said mammal.

In another embodiment, the present invention relates to the use of PRO polypeptides to delay, prevent or rescue retinal cells (including retinal neurons; *e.g.*, photoreceptor cells, other retinal neurons, and supportive cells from injury or death. Other retinal neurons include, but are not limited to retinal ganglion cells, displaced retinal ganglion cells, amacrine cells, displaced amacrine cells, horizontal and bipolar neurons. Supportive cells include, *e.g.*, Müller cells or RPE cells. Additionally, the invention relates to the use of PRO to stimulate the regeneration of such cells. In one aspect, the PRO polypeptide is an active polypeptide which is at least 80% identical to a native sequence PRO molecule.

In yet another embodiment, the present invention relates to the use of PRO polypeptides to treat any condition which results in injury or death of photoreceptor cells or other retinal cells. Examples of such conditions include: retinal detachment; age-related and other maculopathies; photic retinopathies, surgery-induced retinopathies (either mechanically or light-induced), toxic retinopathies including those resulting from foreign bodies in the eye; diabetic retinopathies; retinopathy of prematurity; viral retinopathies such as CMV or HIV retinopathy related to AIDS; uveitis; ischemic retinopathies due to venous or arterial occlusion or other vascular disorder; retinopathies due to trauma or penetrating lesions of the eye; peripheral vitreoretinopathy; and inherited retinal degenerations. Exemplary retinal degenerations include *e.g.*, hereditary spastic paraplegia with retinal degeneration (Kjellin and Barnard-Scholtz syndromes), *retinitis pigmentosa*, Stargardt disease, Usher syndrome (*retinitis pigmentosa* with congenital hearing loss), and Refsum syndrome (*retinitis pigmentosa*, hereditary hearing loss and polyneuropathy). Additional disorders which result in death of retinal neurons include, retinal tears, detachment of the retina and pigment epithelium, degenerative myopia, acute retinal necrosis syndrome (ARN), traumatic chorioretinopathies or contusion (Purtscher's Retinopathy) and edema.

In yet another embodiment, the present invention provides a method of delaying, preventing or rescuing retinal neurons (*e.g.*, photoreceptors) or other retinal cells from injury or death resulting from

disease or injury in a mammal comprising administering a composition comprising a PRO polypeptide and a pharmaceutically-acceptable carrier to said mammal. In one aspect, the composition comprises a therapeutically effective amount of PRO polypeptide. In another aspect, the composition comprises a further reactive ingredient, which may, for example, be a neuronal survival agent. Preferably the composition is sterile. In yet a further aspect, the method comprising contacting said retinal neurons with a therapeutically effective amount of a composition of PRO polypeptide and a pharmaceutically-acceptable carrier. The contact may be effected intraocularly, intravitreally, injection into the subretinal space or by means of an implant. In one aspect, the composition comprises a therapeutically effective amount of PRO. In another, the composition comprises a further active ingredient, which may, for example, be a neuronal survival agent.

In yet another embodiment, the present invention provides articles of manufacture and kits that comprise a PRO polypeptide. The articles of manufacture comprise a container, an instruction on the container, and a composition contained within the container. The instruction on the container may indicate that the compositions can be used to delay, prevent or rescue retinal neurons or other retinal cells from injury or death. The composition may contain an active agent, the active agent may comprise a PRO polypeptide.

In yet another embodiment, the present invention provides a method for delaying or preventing the death of retinal cells in a mammal comprising administering a therapeutically effective amount of: (a) a PRO polypeptide or (b) an agonist of a PRO polypeptide, wherein the death of retinal cells in the mammal is reduced or prevented.

In yet another embodiment, the present invention provides a method for rescuing or preventing the death or injury to retinal cells in a mammal comprising administering a therapeutically effective amount of: (a) a PRO polypeptide; or (b) an agonist of a PRO polypeptide, wherein the death and/or injury to said cells is prevented or reduced.

In yet another embodiment, the present invention provides a method for delaying or preventing the death of retinal neurons comprising contacting said neurons with an effective amount of a PRO175, PRO220, PRO216, PRO306, PRO322, PRO346, PRO840, PRO826, PRO828, PRO1068 or PRO1132 polypeptide.

In yet another aspect, the present invention provides a method for delaying or preventing the death of photoreceptor cells comprising contacting said cells with an effective amount of a PRO175, PRO216, PRO220 or PRO243.

In yet another embodiment, the present invention is directed to a composition of matter useful for the production of a medicament useful for delaying, preventing or rescuing retinal cells (e.g., retinal neurons, photoreceptor cells) from injury or death, wherein the composition comprises a PRO polypeptide.

B. Additional Embodiments

In other embodiments of the present invention, the invention provides vectors comprising DNA encoding any of the herein described polypeptides. Host cell comprising any such vector are also provided. By way of example, the host cells may be CHO cells, *E. coli*, or yeast. A process for producing any of the

herein described polypeptides is further provided and comprises culturing host cells under conditions suitable for expression of the desired polypeptide and recovering the desired polypeptide from the cell culture.

5 In other embodiments, the invention provides chimeric molecules comprising any of the herein described polypeptides fused to a heterologous polypeptide or amino acid sequence. Example of such chimeric molecules comprise any of the herein described polypeptides fused to an epitope tag sequence or a Fc region of an immunoglobulin.

10 In another embodiment, the invention provides an antibody which specifically binds to any of the above or below described polypeptides. Optionally, the antibody is a monoclonal antibody, humanized antibody, antibody fragment or single-chain antibody.

In yet other embodiments, the invention provides oligonucleotide probes useful for isolating genomic and cDNA nucleotide sequences or as antisense probes, wherein those probes may be derived from any of the above or below described nucleotide sequences.

15 In other embodiments, the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence that encodes a PRO polypeptide.

20 In one aspect, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule encoding a PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence lacking the signal peptide as disclosed herein, an extracellular domain of a transmembrane protein, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of the full-length amino acid sequence as disclosed herein, or (b) the complement of the DNA molecule of (a).

25 In other aspects, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity,

alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule comprising the coding sequence of a full-length PRO polypeptide cDNA as disclosed herein, the coding sequence of a PRO polypeptide lacking the signal peptide as disclosed herein, the coding sequence of an extracellular domain of a transmembrane PRO polypeptide, with or without the signal peptide, as disclosed herein or the coding sequence of any other specifically defined fragment of the full-length amino acid sequence as disclosed herein, or (b) the complement of the DNA molecule of (a).

In a further aspect, the invention concerns an isolated nucleic acid molecule comprising a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule that encodes the same mature polypeptide encoded by any of the human protein cDNAs deposited with the ATCC as disclosed herein, or (b) the complement of the DNA molecule of (a).

Another aspect the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a PRO polypeptide which is either transmembrane domain-deleted or transmembrane domain-inactivated, or is complementary to such encoding nucleotide sequence, wherein the transmembrane domain(s) of such polypeptide are disclosed herein. Therefore, soluble extracellular domains of the herein described PRO polypeptides are contemplated.

Another embodiment is directed to fragments of a PRO polypeptide coding sequence, or the complement thereof, that may find use as, for example, hybridization probes, for encoding fragments of a PRO polypeptide that may optionally encode a polypeptide comprising a binding site for an anti-PRO antibody or as antisense oligonucleotide probes. Such nucleic acid fragments are usually at least about 20 nucleotides in length, alternatively at least about 30 nucleotides in length, alternatively at least about 40 nucleotides in length, alternatively at least about 50 nucleotides in length, alternatively at least about 60 nucleotides in length, alternatively at least about 70 nucleotides in length, alternatively at least about 80 nucleotides in length, alternatively at least about 90 nucleotides in length, alternatively at least about 100 nucleotides in length, alternatively at least about 110 nucleotides in length, alternatively at least about 120

nucleotides in length, alternatively at least about 130 nucleotides in length, alternatively at least about 140 nucleotides in length, alternatively at least about 150 nucleotides in length, alternatively at least about 160 nucleotides in length, alternatively at least about 170 nucleotides in length, alternatively at least about 180 nucleotides in length, alternatively at least about 190 nucleotides in length, alternatively at least about 200 nucleotides in length, alternatively at least about 250 nucleotides in length, alternatively at least about 300 nucleotides in length, alternatively at least about 350 nucleotides in length, alternatively at least about 400 nucleotides in length, alternatively at least about 450 nucleotides in length, alternatively at least about 500 nucleotides in length, alternatively at least about 600 nucleotides in length, alternatively at least about 700 nucleotides in length, alternatively at least about 800 nucleotides in length, alternatively at least about 900 nucleotides in length and alternatively at least about 1000 nucleotides in length, wherein in this context the term "about" means the referenced nucleotide sequence length plus or minus 10% of that referenced length. It is noted that novel fragments of a PRO polypeptide-encoding nucleotide sequence may be determined in a routine manner by aligning the PRO polypeptide-encoding nucleotide sequence with other known nucleotide sequences using any of a number of well known sequence alignment programs and determining which PRO polypeptide-encoding nucleotide sequence fragment(s) are novel. All of such PRO polypeptide-encoding nucleotide sequences are contemplated herein. Also contemplated are the PRO polypeptide fragments encoded by these nucleotide molecule fragments, preferably those PRO polypeptide fragments that comprise a binding site for an anti-PRO antibody.

In another embodiment, the invention provides isolated PRO polypeptide encoded by any of the isolated nucleic acid sequences hereinabove identified.

In a certain aspect, the invention concerns an isolated PRO polypeptide, comprising an amino acid sequence having at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86% amino acid sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to a PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence lacking the signal peptide as disclosed herein, an extracellular domain of a transmembrane protein, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of the full-length amino acid sequence as disclosed herein.

In a further aspect, the invention concerns an isolated PRO polypeptide comprising an amino acid sequence having at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least

about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86% amino acid sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to an amino acid sequence encoded by any of the human protein cDNAs deposited with the ATCC as disclosed herein.

In a specific aspect, the invention provides an isolated PRO polypeptide without the N-terminal signal sequence and/or the initiating methionine and is encoded by a nucleotide sequence that encodes such an amino acid sequence as hereinbefore described. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from the cell culture.

Another aspect the invention provides an isolated PRO polypeptide which is either transmembrane domain-deleted or transmembrane domain-inactivated. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from the cell culture.

In yet another embodiment, the invention concerns agonists and antagonists of a native PRO polypeptide as defined herein. In a particular embodiment, the agonist or antagonist is an anti-PRO antibody or a small molecule.

In a further embodiment, the invention concerns a method of identifying agonists or antagonists to a PRO polypeptide which comprise contacting the PRO polypeptide with a candidate molecule and monitoring a biological activity mediated by said PRO polypeptide. Preferably, the PRO polypeptide is a native PRO polypeptide.

In a still further embodiment, the invention concerns a composition of matter comprising a PRO polypeptide, or an agonist or antagonist of a PRO polypeptide as herein described, or an anti-PRO antibody, in combination with a carrier. Optionally, the carrier is a pharmaceutically acceptable carrier.

Another embodiment of the present invention is directed to the use of a PRO polypeptide, or an agonist or antagonist thereof as hereinbefore described, or an anti-PRO antibody, for the preparation of a medicament useful in the treatment of a condition which is responsive to the PRO polypeptide, an agonist or antagonist thereof or an anti-PRO antibody.

Other aspects of the invention will become apparent from the following detailed description and the claims.

Brief Description of the Drawings

- Figure 1 shows DNA19355-1150 (SEQ ID NO:1).
- Figure 2 shows a native sequence PRO175 polypeptide (SEQ ID NO:2).
- Figure 3 shows DNA32298-1132 (SEQ ID NO:7).
- 5 Figure 4 shows a native sequence PRO220 polypeptide (SEQ ID NO:8).
- Figure 5 shows DNA33087-1158 (SEQ ID NO:9).
- Figure 6 shows a native sequence PRO216 polypeptide (SEQ ID NO:10).
- Figure 7 is a pictorial representation of the genomic clones used in the cloning of DNA35917 (SEQ ID NO:14) described in Example 1.
- 10 Figure 8 shows DNA35917-1207 (SEQ ID NO:14).
- Figure 9 shows a native sequence PRO243 polypeptide (SEQ ID NO:15).
- Figure 10 shows DNA39984-1221 (SEQ ID NO:27).
- Figure 11 shows a native sequence PRO306 polypeptide (SEQ ID NO:28).
- Figure 12 shows DNA44167-1243 (SEQ ID NO:30).
- 15 Figure 13 shows a native sequence PRO346 polypeptide (SEQ ID NO:31).
- Figure 14 shows DNA48336-1309 (SEQ ID NO:41).
- Figure 15 shows a native sequence PRO322 polypeptide (SEQ ID NO:42).
- Figure 16 shows DNA49142-1430 (SEQ ID NO:43).
- Figure 17 shows a native sequence PRO536 polypeptide (SEQ ID NO:46).
- 20 Figure 18 shows DNA52192-1369 (SEQ ID NO:49).
- Figure 19 shows a native sequence PRO943 polypeptide (SEQ ID NO:48).
- Figure 20 shows DNA53987-1438 (SEQ ID NO:53).
- Figure 21 shows a native sequence PRO840 polypeptide (SEQ ID NO:54).
- Figure 22 shows DNA57037-1444 (SEQ ID NO:59).
- 25 Figure 23 shows a native sequence PRO828 polypeptide (SEQ ID NO:60).
- Figure 24 shows DNA57694-1341 (SEQ ID NO:64).
- Figure 25 shows a native sequence PRO826 polypeptide (SEQ ID NO:65).
- Figure 26 shows DNA59214-1449 (SEQ ID NO:66).
- Figure 27 shows a native sequence PRO1068 polypeptide (SEQ ID NO:70).
- 30 Figure 28 shows DNA59767-1489 (SEQ ID NO:75).
- Figure 29 shows a native sequence PRO1132 polypeptide (SEQ ID NO:76).

Detailed Description of the Invention**I. Definitions:**

- 35 The terms employed throughout this application are to be construed with the normal meaning to those of ordinary skill in the art. However, applicants desire that the following terms be construed with the particular definitions as described. All references mentioned in this application should be interpreted and read as being incorporated by reference.

The terms "protein" or "polypeptide" are intended to be used interchangeably. They refer to a chain of two (2) or more amino acids which are linked together with peptide or amide bonds, regardless of post-translational modification (e.g., glycosylation or phosphorylation). The polypeptides of this invention may comprise more than one subunit, where each subunit is encoded by a separate DNA sequence.

5 "Active" or "activity" for the purposes herein refers to form(s) of PRO polypeptide which retain a biological and/or an immunological activity of native or naturally-occurring PRO wherein "biological" activity refers to a biological function (either inhibitory or stimulatory) caused by a native or naturally-occurring PRO, respectively, other than the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring PRO. An "immunological" activity refers to
10 the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring PRO polypeptide. Preferably, a biological activity of a PRO polypeptide is the promotion of the survival of, or the delaying or prevention of injury and/or death of retinal cells, e.g., photoreceptor cells or other supportive cells.

"Promoting the survival of", or the "delaying or preventing of injury and/or death of retinal cells",
15 e.g., photoreceptor cells or other supportive cells which occurs as a result of the method of the invention refers to the ability to keep such retinal cells viable or alive for a period of time greater than is observed without application of said method. Retinal cell death can result from injury, disease or even aging. Retinal cell injury can also result in degraded cells or those having a limited capacity for normal physiological operation. The effect can be measured either *in vitro* with isolated retinal cells or *in vivo* with subjects
20 having compromised retinal cells due to injury or disease.

An "ocular disorder" is any condition that would benefit from treatment with the PRO polypeptides. This includes both chronic and acute disorders, as well as those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include any condition which results in injury or death of photoreceptor or other retinal cells. Examples of
25 conditions include: retinal detachment, age-related and other maculopathies, photic retinopathies, surgery-induced retinopathies (either mechanically or light-induced), toxic retinopathies including those resulting from foreign bodies in the eye, diabetic retinopathies, retinopathy of prematurity, viral retinopathies such as CMV or HIV retinopathy related to AIDS, uveitis, ischemic retinopathies due to venous or arterial occlusion or other vascular disorder, retinopathies due to trauma or penetrating lesions of the eye, peripheral vitreoretinopathy, and inherited retinal degenerations. Exemplary retinal degenerations include e.g., hereditary spastic paraplegia with retinal degeneration (Kjellin and Bamard-Scholz syndromes), *retinitis pigmentosa*, Stargardt disease, Usher's syndrome (*retinitis pigmentosa* with congenital hearing loss), and Refsum's syndrome (*retinitis pigmentosa*, hereditary hearing loss, and polyneuropathy). Additional disorders which result in death of retinal neurons include, retinal tears, detachment of the retina and pigment
30 epithelium, degenerative myopia, acute retinal necrosis syndrome (ARN), traumatic chorioretinopathies or contusion (Purtscher's Retinopathy) and edema.

The term "effective amount" is a quantity which is at least the minimum concentration or amount or a PRO polypeptide and/or antagonist/agonist which causes, induces or results in a measurable promotion in the survival or delay or prevention of injury and/or death to retinal cells as measured in an *in vitro* assay.
40 Furthermore, a "therapeutically effective amount" is the minimum concentration or amount or a PRO

polypeptide and/or agonist/antagonist which is required to achieve measurable improvement in the survival of or delay or prevention of injury and/or death to retinal cells which is manifested in an ocular disorder.

A "neuronal survival agent" is an agent, other than the PRO polypeptides described herein, which possesses the ability to delay, prevent or rescue retinal cells from injury or death in a readily quantifiable manner when administered either singularly or in combination with another chemical and/or biological molecule. For example, Vitamin A, antioxidants (e.g., Vitamin E, ascorbate, *etc.*), calcium overload blockers (flunarizine) and growth factors (e.g., aFGF, bFGF, TGF- β , BDNF, NT-4, IGF-I, CNTF, IL-1, NT-3, IGF-II, TNF- α , TNF- β and NGF).

10 II. Additional Definitions

The terms "PRO polypeptide" and "PRO" as used herein and when immediately followed by a numerical designation refer to various polypeptides, wherein the complete designation (*i.e.*, PRO/number) refers to specific polypeptide sequences as described herein. The terms "PRO/number polypeptide" and "PRO/number" wherein the term "number" is provided as an actual numerical designation as used herein encompass native sequence polypeptides and polypeptide variants (which are further defined herein). The PRO polypeptides described herein may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods. The term "PRO polypeptide" refers to each individual PRO/number polypeptide disclosed herein. All disclosures in this specification which refer to the "PRO polypeptide" refer to each of the polypeptides individually as well as jointly. For example, descriptions of the preparation of, purification of, derivation of, formation of antibodies to or against, administration of, compositions containing, treatment of a disease with, *etc.*, pertain to each polypeptide of the invention individually. The term "PRO polypeptide" also includes variants of the PRO/number polypeptides disclosed herein.

A "native sequence PRO polypeptide" comprises a polypeptide having the same amino acid sequence as the corresponding PRO polypeptide derived from nature. Such native sequence PRO polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence PRO polypeptide" specifically encompasses naturally-occurring truncated or secreted forms of the specific PRO polypeptide (e.g., an extracellular domain sequence), naturally-occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants of the polypeptide. In various embodiments of the invention, the native sequence PRO polypeptides disclosed herein are mature or full-length native sequence polypeptides comprising the full-length amino acids sequences shown in the accompanying figures. Start and stop codons are shown in bold font and underlined in the figures. However, while the PRO polypeptide disclosed in the accompanying figures are shown to begin with methionine residues designated herein as amino acid position 1 in the figures, it is conceivable and possible that other methionine residues located either upstream or downstream from the amino acid position 1 in the figures may be employed as the starting amino acid residue for the PRO polypeptides.

The PRO polypeptide "extracellular domain" or "ECD" refers to a form of the PRO polypeptide which is essentially free of the transmembrane and cytoplasmic domains. Ordinarily, a PRO polypeptide ECD will have less than 1% of such transmembrane and/or cytoplasmic domains and preferably, will have less than 0.5% of such domains. It will be understood that any transmembrane domains identified for the

PRO polypeptides of the present invention are identified pursuant to criteria routinely employed in the art for identifying that type of hydrophobic domain. The exact boundaries of a transmembrane domain may vary but most likely by no more than about 5 amino acids at either end of the domain as initially identified herein. Optionally, therefore, an extracellular domain of a PRO polypeptide may contain from about 5 or
5 fewer amino acids on either side of the transmembrane domain/extracellular domain boundary as identified in the Examples or specification and such polypeptides, with or without the associated signal peptide, and nucleic acid encoding them, are contemplated by the present invention.

The approximate location of the "signal peptides" of the various PRO polypeptides disclosed herein are shown in the present specification and/or the accompanying figures. It is noted; however, that
10 the C-terminal boundary of a signal peptide may vary, but most likely by no more than about 5 amino acids on either side of the signal peptide C-terminal boundary as initially identified herein, wherein the C-terminal boundary of the signal peptide may be identified pursuant to criteria routinely employed in the art for identifying that type of amino acid sequence element (e.g., Nielsen *et al.*, *Prot. Eng.* 10:1-6 (1997) and von Heinje *et al.*, *Nucl. Acids. Res.* 14:4683-4690 (1986)). Moreover, it is also recognized that, in some cases,
15 cleavage of a signal sequence from a secreted polypeptide is not entirely uniform, resulting in more than one secreted species. These mature polypeptides, where the signal peptide is cleaved within no more than about 5 amino acids on either side of the C-terminal boundary of the signal peptide as identified herein, and the polynucleotides encoding them, are contemplated by the present invention.

"PRO polypeptide variant" means an active PRO polypeptide as defined above or below having at
20 least about 80% amino acid sequence identity with a full-length native sequence PRO polypeptide sequence as disclosed herein, a PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Such PRO polypeptide variants include, for instance, PRO polypeptides wherein one or more amino acid residues are added, or
25 deleted, at the N- or C-terminus of the full-length native amino acid sequence. Ordinarily, a PRO polypeptide variant will have at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about
30 86% amino acid sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about
35 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to a full-length native sequence PRO polypeptide sequence as disclosed herein, a PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO
40 polypeptide, with or without the signal peptide, as disclosed herein or any other specifically defined

fragment of a full-length PRO polypeptide sequence as disclosed herein. Ordinarily, PRO variant polypeptides are at least about 10 amino acids in length, alternatively at least about 20 amino acids in length, alternatively at least about 30 amino acids in length, alternatively at least about 40 amino acids in length, alternatively at least about 50 amino acids in length, alternatively at least about 60 amino acids in length, alternatively at least about 70 amino acids in length, alternatively at least about 80 amino acids in length, alternatively at least about 90 amino acids in length, alternatively at least about 100 amino acids in length, alternatively at least about 150 amino acids in length, alternatively at least about 200 amino acids in length, alternatively at least about 300 amino acids in length, or more.

"Percent (%) amino acid sequence identity" with respect to the PRO polypeptide sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific PRO polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2, wherein the complete source code for the ALIGN-2 program is provided in Table 1 below. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code shown in Table 1 below has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California or may be compiled from the source code provided in Table 1 below. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. As examples of % amino acid sequence identity calculations using this method, Tables 2 and 3 demonstrate how to calculate the % amino acid sequence identity of the amino acid sequence designated "Comparison Protein" to the amino acid sequence designated "PRO", wherein "PRO"

represents the amino acid sequence of a hypothetical PRO polypeptide of interest, "Comparison Protein" represents the amino acid sequence of a polypeptide against which the "PRO" polypeptide of interest is being compared, and "X," "Y" and "Z" each represent different hypothetical amino acid residues.

Unless specifically stated otherwise, all % amino acid sequence identity values used herein are
5 obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program. However, % amino acid sequence identity values may also be obtained as described below by using the WU-BLAST-2 computer program (Altschul *et al.*, *Methods in Enzymology* 266:460-480 (1996)). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, *i.e.*, the adjustable parameters, are set with the following values: overlap span = 1, overlap fraction = 0.125, word
10 threshold (T) = 11, and scoring matrix = BLOSUM62. When WU-BLAST-2 is employed, a % amino acid sequence identity value is determined by dividing (a) the number of matching identical amino acid residues between the amino acid sequence of the PRO polypeptide of interest having a sequence derived from the native PRO polypeptide and the comparison amino acid sequence of interest (*i.e.*, the sequence against which the PRO polypeptide of interest is being compared which may be a PRO variant polypeptide) as
15 determined by WU-BLAST-2 by (b) the total number of amino acid residues of the PRO polypeptide of interest. For example, in the statement "a polypeptide comprising an the amino acid sequence A which has or having at least 80% amino acid sequence identity to the amino acid sequence B", the amino acid sequence A is the comparison amino acid sequence of interest and the amino acid sequence B is the amino acid sequence of the PRO polypeptide of interest.

20 Percent amino acid sequence identity may also be determined using the sequence comparison program NCBI-BLAST2 (Altschul *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997)). The NCBI-BLAST2 sequence comparison program may be downloaded from <http://www.ncbi.nlm.nih.gov> or otherwise obtained from the National Institute of Health, Bethesda, MD. NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand
25 = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

In situations where NCBI-BLAST2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a
30 certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

35 where X is the number of amino acid residues scored as identical matches by the sequence alignment program NCBI-BLAST2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A.

"PRO variant polynucleotide" or "PRO variant nucleic acid sequence" means a nucleic acid molecule which encodes an active PRO polypeptide as defined below and which has at least about 80% nucleic acid sequence identity with a nucleotide acid sequence encoding a full-length native sequence PRO polypeptide sequence as disclosed herein, a full-length native sequence PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Ordinarily, a PRO variant polynucleotide will have at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity with a nucleic acid sequence encoding a full-length native sequence PRO polypeptide sequence as disclosed herein, a full-length native sequence PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal sequence, as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Variants do not encompass the native nucleotide sequence.

Ordinarily, PRO variant polynucleotides are at least about 30 nucleotides in length, alternatively at least about 60 nucleotides in length, alternatively at least about 90 nucleotides in length, alternatively at least about 120 nucleotides in length, alternatively at least about 150 nucleotides in length, alternatively at least about 180 nucleotides in length, alternatively at least about 210 nucleotides in length, alternatively at least about 240 nucleotides in length, alternatively at least about 270 nucleotides in length, alternatively at least about 300 nucleotides in length, alternatively at least about 450 nucleotides in length, alternatively at least about 600 nucleotides in length, alternatively at least about 900 nucleotides in length, or more.

"Percent (%) nucleic acid sequence identity" with respect to PRO-encoding nucleic acid sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in the PRO nucleic acid sequence of interest, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. For purposes herein, however, % nucleic acid sequence identity values are generated using the sequence comparison computer program ALIGN-2, wherein the complete source code for the ALIGN-2 program is provided in Table 1 below. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code shown in Table 1 below has been

filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California or may be compiled from the source code provided in Table 1 below. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for nucleic acid sequence comparisons, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows:

$$100 \text{ times the fraction } W/Z$$

where W is the number of nucleotides scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of C and D, and where Z is the total number of nucleotides in D. It will be appreciated that where the length of nucleic acid sequence C is not equal to the length of nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C. As examples of % nucleic acid sequence identity calculations, Tables 4 and 5, demonstrate how to calculate the % nucleic acid sequence identity of the nucleic acid sequence designated "Comparison DNA" to the nucleic acid sequence designated "PRO-DNA", wherein "PRO-DNA" represents a hypothetical PRO-encoding nucleic acid sequence of interest, "Comparison DNA" represents the nucleotide sequence of a nucleic acid molecule against which the "PRO-DNA" nucleic acid molecule of interest is being compared, and "N", "L" and "V" each represent different hypothetical nucleotides.

Unless specifically stated otherwise, all % nucleic acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program. However, % nucleic acid sequence identity values may also be obtained as described below by using the WU-BLAST-2 computer program (Altschul *et al.*, *Methods in Enzymology* 266:460-480 (1996)). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, i.e., the adjustable parameters, are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11, and scoring matrix = BLOSUM62. When WU-BLAST-2 is employed, a % nucleic acid sequence identity value is determined by dividing (a) the number of matching identical nucleotides between the nucleic acid sequence of the PRO polypeptide-encoding nucleic acid molecule of interest having a sequence derived from the native sequence PRO polypeptide-encoding nucleic acid and the comparison nucleic acid molecule of interest (i.e., the sequence against which the PRO polypeptide-encoding nucleic acid molecule of interest is being compared which may be a variant PRO polynucleotide) as determined by WU-BLAST-2 by (b) the total number of nucleotides of the PRO polypeptide-encoding nucleic acid molecule of interest. For example, in the statement "an isolated nucleic acid molecule comprising a nucleic acid sequence A which has or having at least 80% nucleic acid sequence identity to the nucleic acid sequence B", the nucleic acid sequence A is the comparison nucleic acid molecule of interest and the nucleic

acid sequence B is the nucleic acid sequence of the PRO polypeptide-encoding nucleic acid molecule of interest.

Percent nucleic acid sequence identity may also be determined using the sequence comparison program NCBI-BLAST2 (Altschul *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997)). The NCBI-BLAST2 sequence comparison program may be downloaded from <http://www.ncbi.nlm.nih.gov> or otherwise obtained from the National Institute of Health, Bethesda, MD. NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

In situations where NCBI-BLAST2 is employed for sequence comparisons, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows:

$$100 \text{ times the fraction } W/Z$$

where W is the number of nucleotides scored as identical matches by the sequence alignment program NCBI-BLAST2 in that program's alignment of C and D, and where Z is the total number of nucleotides in D. It will be appreciated that where the length of nucleic acid sequence C is not equal to the length of nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C.

In other embodiments, PRO variant polynucleotides are nucleic acid molecules that encode an active PRO polypeptide and which are capable of hybridizing, preferably under stringent hybridization and wash conditions, to nucleotide sequences encoding a full-length PRO polypeptide as disclosed herein. PRO variant polypeptides may be those that are encoded by a PRO variant polynucleotide.

"Isolated," when used to describe the various polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the PRO polypeptide natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

An "isolated" PRO polypeptide-encoding nucleic acid or other polypeptide-encoding nucleic acid is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the polypeptide-encoding nucleic acid. An isolated polypeptide-encoding nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated polypeptide-encoding nucleic acid molecules therefore are distinguished from

the specific polypeptide-encoding nucleic acid molecule as it exists in natural cells. However, an isolated polypeptide-encoding nucleic acid molecule includes polypeptide-encoding nucleic acid molecules contained in cells that ordinarily express the polypeptide where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

5 The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

10 Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, 15 in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

20 The term "antibody" is used in the broadest sense and specifically covers, for example, single anti-PRO monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies), anti-PRO antibody compositions with polyepitopic specificity, single chain anti-PRO antibodies, and fragments of anti-PRO antibodies (see below). The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

25 "Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The 30 higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel *et al.*, *Current Protocols in Molecular Biology*, Wiley Interscience Publishers, (1995).

35 "Stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM 40 sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl,

0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

5 "Moderately stringent conditions" may be identified as described by Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and %SDS) less stringent than those described above. An example of moderately stringent conditions is overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

15 The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising a PRO polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

20 As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (*i.e.*, is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

30 "Active" or "activity" for the purposes herein refers to form(s) of a PRO polypeptide which retain a biological and/or an immunological activity of native or naturally-occurring PRO, wherein "biological" activity refers to a biological function (either inhibitory or stimulatory) caused by a native or naturally-occurring PRO other than the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring PRO and an "immunological" activity refers to the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring PRO.

35 The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native PRO polypeptide disclosed herein. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics a biological activity of a native PRO polypeptide disclosed herein. Suitable agonist or antagonist molecules

specifically include agonist or antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native PRO polypeptides, peptides, antisense oligonucleotides, small organic molecules, etc. Methods for identifying agonists or antagonists of a PRO polypeptide may comprise contacting a PRO polypeptide with a candidate agonist or antagonist molecule and measuring a detectable change in one or more biological activities normally associated with the PRO polypeptide.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats, rabbits, etc. Preferably, the mammal is human.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies (Zapata *et al.*, *Protein Eng.* 8(10): 1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen-

binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

5 The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')₂ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments
10 are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains.

15 Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2.

"Single-chain Fv" or "sFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).
20

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H-V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).
25

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain.
30 Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.
35

An antibody that "specifically binds to" or is "specific for" a particular polypeptide or an epitope on a particular polypeptide is one that binds to that particular polypeptide or epitope on a particular polypeptide without substantially binding to any other polypeptide or polypeptide epitope.

5 The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (*e.g.* radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

10 By "solid phase" is meant a non-aqueous matrix to which the antibody of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (*e.g.*, controlled pore glass), polysaccharides (*e.g.*, agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (*e.g.*, an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

15 A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as a PRO polypeptide or antibody thereto) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

A "small molecule" is defined herein to have a molecular weight below about 500 Daltons.

Table 1

```

/*
 *
 * C-C increased from 12 to 15
5  * Z is average of EQ
 * B is average of ND
 * match with stop is _M; stop-stop = 0; J (joker) match = 0
 */
10 #define _M      -8      /* value of a match with a stop */

int _day[26][26] = {
/*  A B C D E F G H I J K L M N O P Q R S T U V W X Y Z */
/* A */ { 2, 0, -2, 0, 0, -4, 1, -1, -1, 0, -1, -2, -1, 0, _M, 1, 0, -2, 1, 1, 0, 0, -6, 0, -3, 0},
/* B */ { 0, 3, -4, 3, 2, -5, 0, 1, -2, 0, 0, -3, -2, 2, _M, -1, 1, 0, 0, 0, 0, -2, -5, 0, -3, 1},
15 /* C */ {-2, -4, 15, -5, -5, -4, -3, -2, 0, -5, -6, -5, -4, _M, -3, -5, -4, 0, -2, 0, -2, -8, 0, 0, -5},
/* D */ { 0, 3, -5, 4, 3, -6, 1, 1, -2, 0, 0, -4, -3, 2, _M, -1, 2, -1, 0, 0, 0, -2, -7, 0, -4, 2},
/* E */ { 0, 2, -5, 3, 4, -5, 0, 1, -2, 0, 0, -3, -2, 1, _M, -1, 2, -1, 0, 0, 0, -2, -7, 0, -4, 3},
/* F */ {-4, -5, -4, -6, -5, 9, -5, -2, 1, 0, -5, 2, 0, -4, _M, -5, -5, -4, -3, -3, 0, -1, 0, 0, 7, -5},
/* G */ { 1, 0, -3, 1, 0, -5, 5, -2, -3, 0, -2, -4, -3, 0, _M, -1, -1, -3, 1, 0, 0, -1, -7, 0, -5, 0},
20 /* H */ {-1, 1, -3, 1, 1, -2, -2, 6, -2, 0, 0, -2, -2, 2, _M, 0, 3, 2, -1, -1, 0, -2, -3, 0, 0, 2},
/* I */ {-1, -2, -2, -2, 2, 1, -3, -2, 5, 0, -2, 2, 2, -2, _M, -2, -2, -2, -1, 0, 0, 4, -5, 0, -1, -2},
/* J */ { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, _M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* K */ {-1, 0, -5, 0, 0, -5, -2, 0, -2, 0, 5, -3, 0, 1, _M, -1, 1, 3, 0, 0, 0, -2, -3, 0, -4, 0},
/* L */ {-2, -3, -6, -4, -3, 2, -4, -2, 2, 0, -3, 6, 4, -3, _M, -3, -2, -3, -3, -1, 0, 2, -2, 0, -1, -2},
25 /* M */ {-1, -2, -5, -3, -2, 0, -3, -2, 2, 0, 0, 4, 6, -2, _M, -2, -1, 0, -2, -1, 0, 2, -4, 0, -2, -1},
/* N */ { 0, 2, -4, 2, 1, -4, 0, 2, -2, 0, 1, -3, -2, 2, _M, -1, 1, 0, 1, 0, 0, -2, -4, 0, -2, 1},
/* O */ { _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, 0, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M},
/* P */ { 1, -1, -3, -1, -1, -5, -1, 0, -2, 0, -1, -3, -2, -1, _M, 6, 0, 0, 1, 0, 0, -1, -6, 0, -5, 0},
/* Q */ { 0, 1, -5, 2, 2, -5, -1, 3, -2, 0, 1, -2, -1, 1, _M, 0, 4, 1, -1, -1, 0, -2, -5, 0, -4, 3},
30 /* R */ {-2, 0, -4, -1, -1, -4, -3, 2, -2, 0, 3, -3, 0, 0, _M, 0, 1, 6, 0, -1, 0, -2, 2, 0, -4, 0},
/* S */ { 1, 0, 0, 0, 0, -3, 1, -1, -1, 0, 0, -3, -2, 1, _M, 1, -1, 0, 2, 1, 0, -1, -2, 0, -3, 0},
/* T */ { 1, 0, -2, 0, 0, -3, 0, -1, 0, 0, 0, -1, -1, 0, _M, 0, -1, -1, 1, 3, 0, 0, -5, 0, -3, 0},
/* U */ { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, _M, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* V */ { 0, -2, -2, -2, -2, -1, -1, -2, 4, 0, -2, 2, 2, -2, _M, -1, -2, -2, -1, 0, 0, 4, -6, 0, -2, -2},
35 /* W */ {-6, -5, -8, -7, -7, 0, -7, -3, -5, 0, -3, -2, -4, -4, _M, -6, -5, 2, -2, -5, 0, -6, 17, 0, 0, -6},
/* X */ { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, _M, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* Y */ {-3, -3, 0, -4, -4, 7, -5, 0, -1, 0, -4, -1, -2, -2, _M, -5, -4, -4, -3, -3, 0, -2, 0, 0, 10, -4},
/* Z */ { 0, 1, -5, 2, 3, -5, 0, 2, -2, 0, 0, -2, -1, 1, _M, 0, 3, 0, 0, 0, -2, -6, 0, -4, 4}
};
40
45
50

```

Table 1 (cont')

```

/*
*/
5  #include <stdio.h>
   #include <ctype.h>

   #define MAXJMP      16      /* max jumps in a diag */
   #define MAXGAP      24      /* don't continue to penalize gaps larger than this */
10  #define JMPS        1024    /* max jmps in an path */
   #define MX          4       /* save if there's at least MX-1 bases since last jmp */

   #define DMAT         3       /* value of matching bases */
   #define DMIS         0       /* penalty for mismatched bases */
15  #define DINS0        8       /* penalty for a gap */
   #define DINS1        1       /* penalty per base */
   #define PINS0        8       /* penalty for a gap */
   #define PINS1        4       /* penalty per residue */

20  struct jmp {
      short             n[MAXJMP]; /* size of jmp (neg for dely) */
      unsigned short    x[MAXJMP]; /* base no. of jmp in seq x */
   }; /* limits seq to 2^16 -1 */

25  struct diag {
      int               score;      /* score at last jmp */
      long              offset;     /* offset of prev block */
      short             ijmp;       /* current jmp index */
      struct jmp        jp;         /* list of jmps */
30  };

   struct path {
      int               spc;         /* number of leading spaces */
      short             n[JMPS]; /* size of jmp (gap) */
35  int               x[JMPS]; /* loc of jmp (last elem before gap) */
   };

   char               *ofile;      /* output file name */
   char               *namex[2];   /* seq names: getseqs() */
40  char               *prog;       /* prog name for err msgs */
   char               *seqx[2];    /* seqs: getseqs() */
   int                dmax;        /* best diag: nw() */
   int                dmax0;       /* final diag */
   int                dna;         /* set if dna: main() */
45  int                endgaps;     /* set if penalizing end gaps */
   int                gapx, gapy;   /* total gaps in seqs */
   int                len0, len1;   /* seq lens */
   int                ngapx, ngapy; /* total size of gaps */
   int                smax;        /* max score: nw() */
50  int                *xbm;        /* bitmap for matching */
   long              offset;       /* current offset in jmp file */
   struct            diag        *dx; /* holds diagonals */
   struct            path        pp[2]; /* holds path for seqs */

55  char               *calloc(), *malloc(), *index(), *strcpy();
   char               *getseq(), *g_calloc();

```

60

Table 1 (cont')

```

/* Needleman-Wunsch alignment program
*
5  * usage: progs file1 file2
*   where file1 and file2 are two dna or two protein sequences.
*   The sequences can be in upper- or lower-case and may contain ambiguity
*   Any lines beginning with ';', '>' or '<' are ignored
10  * Max file length is 65535 (limited by unsigned short x in the jmp struct)
*   A sequence with 1/3 or more of its elements ACGTU is assumed to be DNA
*   Output is in the file "align.out"
*
* The program may create a tmp file in /tmp to hold info about traceback.
* Original version developed under BSD 4.3 on a vax 8650
15 */
#include "nw.h"
#include "day.h"

static  _dbval[26] = {
20      1,14,2,13,0,0,4,11,0,0,12,0,3,15,0,0,0,5,6,8,8,7,9,0,10,0
};

static  _pbval[26] = {
25      1, 2|(1<<('D'-'A'))|(1<<('N'-'A')), 4, 8, 16, 32, 64,
128, 256, 0xFFFFFFFF, 1<<10, 1<<11, 1<<12, 1<<13, 1<<14,
1<<15, 1<<16, 1<<17, 1<<18, 1<<19, 1<<20, 1<<21, 1<<22,
1<<23, 1<<24, 1<<25|(1<<('E'-'A'))|(1<<('Q'-'A'))
};

30  main(ac, av)
      main
      int      ac;
      char      *av[];
{
35      prog = av[0];
      if (ac != 3) {
          fprintf(stderr, "usage: %s file1 file2\n", prog);
          fprintf(stderr, "where file1 and file2 are two dna or two protein sequences.\n");
          fprintf(stderr, "The sequences can be in upper- or lower-case\n");
          fprintf(stderr, "Any lines beginning with ';' or '<' are ignored\n");
          fprintf(stderr, "Output is in the file \"align.out\"\n");
          exit(1);
      }
45      namex[0] = av[1];
      namex[1] = av[2];
      seqx[0] = getseq(namex[0], &len0);
      seqx[1] = getseq(namex[1], &len1);
      xbm = (dna)? _dbval : _pbval;

50      endgaps = 0;
      ofile = "align.out";
      /* 1 to penalize endgaps */
      /* output file */

      nw();
      readjumps();
      print();
55      /* fill in the matrix, get the possible jumps */
      /* get the actual jumps */
      /* print stats, alignment */

      cleanup();
      /* unlink any tmp files */
}

60

```

Table 1 (cont')

```

/* do the alignment, return best score: main()
 * dna: values in Fitch and Smith, PNAS, 80, 1382-1386, 1983
5  * pro: PAM 250 values
 * When scores are equal, we prefer mismatches to any gap, prefer
 * a new gap to extending an ongoing gap, and prefer a gap in seqx
 * to a gap in seq y.
 */
10 nw()
    nw
    {
        char      *px, *py;      /* seqs and ptrs */
        int        *ndely, *dely; /* keep track of dely */
        int        ndelx, delx;   /* keep track of delx */
        int        *tmp;         /* for swapping row0, row1 */
        int        mis;          /* score for each type */
        int        ins0, ins1;    /* insertion penalties */
        register   id;           /* diagonal index */
        register   ij;           /* jmp index */
        register   *col0, *col1; /* score for curr, last row */
        register   xx, yy;       /* index into seqs */

        dx = (struct diag *)g_calloc("to get diags", len0+len1+1, sizeof(struct diag));

25        ndely = (int *)g_calloc("to get ndely", len1+1, sizeof(int));
        dely = (int *)g_calloc("to get dely", len1+1, sizeof(int));
        col0 = (int *)g_calloc("to get col0", len1+1, sizeof(int));
        col1 = (int *)g_calloc("to get col1", len1+1, sizeof(int));
        ins0 = (dna)? DINS0 : PINS0;
        ins1 = (dna)? DINS1 : PINS1;

        smax = -10000;
        if (endgaps) {
35             for (col0[0] = dely[0] = -ins0, yy = 1; yy <= len1; yy++) {
                 col0[yy] = dely[yy] = col0[yy-1] - ins1;
                 ndely[yy] = yy;
             }
             col0[0] = 0;      /* Waterman Bull Math Biol 84 */
40         }
        else
            for (yy = 1; yy <= len1; yy++)
                dely[yy] = -ins0;

45        /* fill in match matrix
        */
        for (px = seqx[0], xx = 1; xx <= len0; px++, xx++) {
            /* initialize first entry in col
            */
50             if (endgaps) {
                 if (xx == 1)
                     col1[0] = delx = -(ins0+ins1);
                 else
                     col1[0] = delx = col0[0] - ins1;
55                 ndelx = xx;
             }
            else {
                 col1[0] = 0;
                 delx = -ins0;
60                 ndelx = 0;
            }
        }
    }

```

Table 1 (cont')

```

5      for (py = seqx[1], yy = 1; yy <= len1; py++, yy++) {          ...nw
      mis = col0[yy-1];
      if (dna)
          mis += (xbm[*px-'A']&xbm[*py-'A'])? DMAT : DMIS;
      else
10         mis += _day[*px-'A'][*py-'A'];

      /* update penalty for del in x seq;
      * favor new del over ongoing del
      * ignore MAXGAP if weighting endgaps
      */
15     if (endgaps || ndely[yy] < MAXGAP) {
        if (col0[yy] - ins0 >= dely[yy]) {
            dely[yy] = col0[yy] - (ins0+ins1);
            ndely[yy] = 1;
20         } else {
            dely[yy] -= ins1;
            ndely[yy]++;
        }
    } else {
25         if (col0[yy] - (ins0+ins1) >= dely[yy]) {
            dely[yy] = col0[yy] - (ins0+ins1);
            ndely[yy] = 1;
        } else
            ndely[yy]++;
30     }

    /* update penalty for del in y seq;
    * favor new del over ongoing del
    */
35     if (endgaps || ndelx < MAXGAP) {
        if (col1[yy-1] - ins0 >= delx) {
            delx = col1[yy-1] - (ins0+ins1);
            ndelx = 1;
40         } else {
            delx -= ins1;
            ndelx++;
        }
    } else {
45         if (col1[yy-1] - (ins0+ins1) >= delx) {
            delx = col1[yy-1] - (ins0+ins1);
            ndelx = 1;
        } else
            ndelx++;
50     }

    /* pick the maximum score; we're favoring
    * mis over any del and delx over dely
    */
55
60

```


Table 1 (cont')

```

id = xx - yy + len1 - 1;
...nw
5      if (mis >= delx && mis >= dely[yy])
        col1[yy] = mis;
      else if (delx >= dely[yy]) {
        col1[yy] = delx;
        ij = dx[id].ijmp;
10      if (dx[id].jp.n[0] && (!dna || (ndelx >= MAXJMP
        && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
          dx[id].ijmp++;
          if (++ij >= MAXJMP) {
15              writejumps(id);
              ij = dx[id].ijmp = 0;
              dx[id].offset = offset;
              offset += sizeof(struct jmp) + sizeof(offset);
          }
        }
        dx[id].jp.n[ij] = ndelx;
        dx[id].jp.x[ij] = xx;
        dx[id].score = delx;
      }
20      else {
        col1[yy] = dely[yy];
        ij = dx[id].ijmp;
        if (dx[id].jp.n[0] && (!dna || (ndely[yy] >= MAXJMP
25        && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
          dx[id].ijmp++;
          if (++ij >= MAXJMP) {
30              writejumps(id);
              ij = dx[id].ijmp = 0;
              dx[id].offset = offset;
              offset += sizeof(struct jmp) + sizeof(offset);
          }
        }
        dx[id].jp.n[ij] = -ndely[yy];
        dx[id].jp.x[ij] = xx;
        dx[id].score = dely[yy];
40      }
      if (xx == len0 && yy < len1) {
        /* last col
        */
        if (endgaps)
45          col1[yy] -= ins0+ins1*(len1-yy);
        if (col1[yy] > smax) {
          smax = col1[yy];
          dmax = id;
        }
      }
50    }
    if (endgaps && xx < len0)
      col1[yy-1] -= ins0+ins1*(len0-xx);
    if (col1[yy-1] > smax) {
55      smax = col1[yy-1];
      dmax = id;
    }
    tmp = col0; col0 = col1; col1 = tmp;
60  }
  (void) free((char *)ndely);
  (void) free((char *)dely);
  (void) free((char *)col0);
  (void) free((char *)col1);
}

```

Table 1 (cont')

```

/*
 *
5  * print() -- only routine visible outside this module
 *
 * static:
 * getmat() -- trace back best path, count matches: print()
 * pr_align() -- print alignment of described in array p[]: print()
10 * dumpblock() -- dump a block of lines with numbers, stars: pr_align()
 * nums() -- put out a number line: dumpblock()
 * putline() -- put out a line (name, [num], seq, [num]): dumpblock()
 * stars() -- put a line of stars: dumpblock()
15 * stripname() -- strip any path and prefix from a seqname
 */

#include "nw.h"

#define SPC      3
20 #define P_LINE 256 /* maximum output line */
#define P_SPC    3 /* space between name or num and seq */

extern _day[26][26];
int olen; /* set output line length */
25 FILE *fx; /* output file */

print()
{
    print
30 {
        int lx, ly, firstgap, lastgap; /* overlap */

        if ((fx = fopen(ofile, "w")) == 0) {
            fprintf(stderr, "%s: can't write %s\n", prog, ofile);
            cleanup(1);
35         }
        fprintf(fx, "<first sequence: %s (length = %d)\n", namex[0], len0);
        fprintf(fx, "<second sequence: %s (length = %d)\n", namex[1], len1);
        olen = 60;
        lx = len0;
        ly = len1;
        firstgap = lastgap = 0;
        if (dmax < len1 - 1) { /* leading gap in x */
            pp[0].spc = firstgap = len1 - dmax - 1;
            ly -= pp[0].spc;
45         }
        else if (dmax > len1 - 1) { /* leading gap in y */
            pp[1].spc = firstgap = dmax - (len1 - 1);
            lx -= pp[1].spc;
        }
        if (dmax0 < len0 - 1) { /* trailing gap in x */
            lastgap = len0 - dmax0 - 1;
            lx -= lastgap;
50         }
        else if (dmax0 > len0 - 1) { /* trailing gap in y */
            lastgap = dmax0 - (len0 - 1);
            ly -= lastgap;
55         }
        getmat(lx, ly, firstgap, lastgap);
        pr_align();
60     }

```

Table 1 (cont')

```

/*
5  * trace back the best path, count matches
*/
static
getmat(lx, ly, firstgap, lastgap)
    getmat
10     int      lx, ly;          /* "core" (minus endgaps) */
    int      firstgap, lastgap; /* leading trailing overlap */
{
    int      nm, i0, i1, siz0, siz1;
    char      outx[32];
    double    pct;
15     register n0, n1;
    register char *p0, *p1;

    /* get total matches, score
    */
20     i0 = i1 = siz0 = siz1 = 0;
    p0 = seqx[0] + pp[1].spc;
    p1 = seqx[1] + pp[0].spc;
    n0 = pp[1].spc + 1;
    n1 = pp[0].spc + 1;

25     nm = 0;
    while ( *p0 && *p1 ) {
        if (siz0) {
            p1++;
            n1++;
            siz0--;
        }
        else if (siz1) {
            p0++;
            n0++;
            siz1--;
        }
        else {
40             if (xbm[*p0-'A']&xbm[*p1-'A'])
                nm++;
            if (n0++ == pp[0].x[i0])
                siz0 = pp[0].n[i0++];
            if (n1++ == pp[1].x[i1])
                siz1 = pp[1].n[i1++];
45             p0++;
            p1++;
        }
    }

50     /* pct homology:
    * if penalizing endgaps, base is the shorter seq
    * else, knock off overhangs and take shorter core
    */
    if (endgaps)
55         lx = (len0 < len1)? len0 : len1;
    else
        lx = (lx < ly)? lx : ly;
    pct = 100.*(double)nm/(double)lx;
    fprintf(fx, "\n");
60     fprintf(fx, "< %d match %s in an overlap of %d: %.2f percent similarity\n",
        nm, (nm == 1)? "" : "es", lx, pct);

```

Table 1 (cont')

```

5      fprintf(fx, "< gaps in first sequence: %d", gapx);
      if (gapx) {
          (void) sprintf(outx, " (%d %s%s)",
              ngapx, (dna)? "base":"residue", (ngapx == 1)? "" : "s");
          fprintf(fx, "%s", outx);

10      fprintf(fx, ", gaps in second sequence: %d", gapy);
      if (gapy) {
          (void) sprintf(outx, " (%d %s%s)",
              ngapy, (dna)? "base":"residue", (ngapy == 1)? "" : "s");
          fprintf(fx, "%s", outx);
      }
15      if (dna)
          fprintf(fx,
              "\n<score: %d (match = %d, mismatch = %d, gap penalty = %d + %d per base)\n",
              smax, DMAT, DMIS, DINSO, DINSI);
      else
20      fprintf(fx,
              "\n<score: %d (Dayhoff PAM 250 matrix, gap penalty = %d + %d per residue)\n",
              smax, PINSO, PINSI);
      if (endgaps)
25      fprintf(fx,
              "<endgaps penalized. left endgap: %d %s%s, right endgap: %d %s%s\n",
              firstgap, (dna)? "base" : "residue", (firstgap == 1)? "" : "s",
              lastgap, (dna)? "base" : "residue", (lastgap == 1)? "" : "s");
      else
30      fprintf(fx, "<endgaps not penalized\n");

      static      nm;          /* matches in core -- for checking */
      static      lmax;        /* lengths of stripped file names */
      static      ij[2];       /* jmp index for a path */
35      static      nc[2];       /* number at start of current line */
      static      ni[2];       /* current elem number -- for gapping */
      static      siz[2];
      static char  *ps[2];      /* ptr to current element */
      static char  *po[2];      /* ptr to next output char slot */
40      static char  out[2][P_LINE]; /* output line */
      static char  star[P_LINE]; /* set by stars() */

      /*
45      * print alignment of described in struct path pp[]
      */
      static
      pr_align()
      {
          int      nn;          /* char count */
50      int      more;
      register    i;

          for (i = 0, lmax = 0; i < 2; i++) {
75      nn = stripname(namex[i]);
          if (nn > lmax)
              lmax = nn;

          nc[i] = 1;
          ni[i] = 1;
60      siz[i] = ij[i] = 0;
          ps[i] = seqx[i];
          po[i] = out[i];
      }

```

...getmat

pr_align

Table 1 (cont')

```

5      for (nn = nm = 0, more = 1; more; ) {
        for (i = more = 0; i < 2; i++) {
            /*
            * do we have more of this sequence?
            */
            if (!*ps[i])
10                continue;

            more++;

            if (pp[i].spc) { /* leading space */
                *po[i]++ = ' ';
15                pp[i].spc--;
            }
            else if (siz[i]) { /* in a gap */
                *po[i]++ = '-';
                siz[i]--;
20            }
            else { /* we're putting a seq element
                    */
                *po[i] = *ps[i];
                if (islower(*ps[i]))
25                    *ps[i] = toupper(*ps[i]);
                po[i]++;
                ps[i]++;

                /*
                * are we at next gap for this seq?
                */
                if (ni[i] == pp[i].x[ij[i]]) {
                    /*
                    * we need to merge all gaps
                    * at this location
                    */
                    siz[i] = pp[i].n[ij[i] + +];
                    while (ni[i] == pp[i].x[ij[i]])
40                        siz[i] += pp[i].n[ij[i] + +];
                }
                ni[i]++;
            }
        }
        if (++nn == olen || !more && nn) {
45            dumpblock();
            for (i = 0; i < 2; i++)
                po[i] = out[i];
            nn = 0;
        }
50    }

    /*
    * dump a block of lines, including numbers, stars: pr_align()
    */
55    static
    dumpblock()
    {
        dumpblock
    {
60        register i;

        for (i = 0; i < 2; i++)
            *po[i]-- = '\0';
    }
}

```

...pr_align

Table 1 (cont')

```

5      (void) putc('\n', fx);
      for (i = 0; i < 2; i++) {
          if (*out[i] && (*out[i] != ' ' || *(po[i]) != ' ')) {
              if (i == 0)
                  nums(i);
              if (i == 0 && *out[i])
                  stars();
              putline(i);
              if (i == 0 && *out[i])
                  fprintf(fx, star);
              if (i == 1)
                  nums(i);
          }
      }
}

20  /*
    * put out a number line: dumpblock()
    */
    static
    nums(ix)
25      {
          int ix; /* index in out[] holding seq line */

          char nline[P_LINE];
          register i, j;
          register char *pn, *px, *py;

          for (pn = nline, i = 0; i < lmax + P_SPC; i++, pn++)
              *pn = ' ';
          for (i = nc[ix], py = out[ix]; *py; py++, pn++) {
35              if (*py == ' ' || *py == '-')
                  *pn = ' ';
              else {
                  if (i % 10 == 0 || (i == 1 && nc[ix] != 1)) {
40                      j = (i < 0)? -i : i;
                      for (px = pn; j /= 10, px--)
                          *px = j % 10 + '0';
                      if (i < 0)
                          *px = '-';
                  }
                  else
45                      *pn = ' ';
                  i++;
              }
          }
          *pn = '\0';
          nc[ix] = i;
          for (pn = nline; *pn; pn++)
              (void) putc(*pn, fx);
          (void) putc('\n', fx);
55      }

    /*
    * put out a line (name, [num], seq, [num]): dumpblock()
    */
    static
    putline(ix)
60      {
          int ix;

```

Table 1 (cont')

...putline

```

5      int          i;
      register char *px;

      for (px = namex[ix], i = 0; *px && *px != ':'; px++, i++)
          (void) putc(*px, fx);
10     for (; i < lmax+P_SPC; i++)
          (void) putc(' ', fx);

      /* these count from 1:
       * ni[] is current element (from 1)
       * nc[] is number at start of current line
       */
15     for (px = out[ix]; *px; px++)
          (void) putc(*px&0x7F, fx);
      (void) putc('\n', fx);
20 }

/*
 * put a line of stars (seqs always in out[0], out[1]): dumpblock()
 */
25 static
stars()
{
    int          i;
30     register char *p0, *p1, cx, *px;

    if (!*out[0] || (*out[0] == ' ' && *(p0[0]) == ' ') ||
        !*out[1] || (*out[1] == ' ' && *(p0[1]) == ' '))
        return;
35     px = star;
    for (i = lmax+P_SPC; i; i--)
        *px++ = ' ';

    for (p0 = out[0], p1 = out[1]; *p0 && *p1; p0++, p1++) {
40         if (isalpha(*p0) && isalpha(*p1)) {

                if (xbm[*p0-'A']&xbm[*p1-'A']) {
                    cx = '*';
                    nm++;
45                 }
                else if (!dna && _day[*p0-'A'][*p1-'A'] > 0)
                    cx = '.';
                else
                    cx = ' ';
50             }
            else
                cx = ' ';

            *px++ = cx;
55         }
        *px++ = '\n';
        *px = '\0';
    }
60 }

```

Table 1 (cont')

```

/*
 * strip path or prefix from pn, return len: pr_align()
 */
5 static
  stripname(pn)
    stripname
    char *pn; /* file name (may be path) */
10 {
    register char *px, *py;

    py = 0;
    for (px = pn; *px; px++)
15         if (*px == '/')
            py = px + 1;
    if (py)
        (void) strcpy(pn, py);
    return(strlen(pn));
20 }

25

30

35

40

45

50

55

60

```


Table 1 (cont')

```

/*
 * cleanup() -- cleanup any tmp file
5  * getseq() -- read in seq, set dna, len, maxlen
 * g_calloc() -- calloc() with error checkin
 * readjumps() -- get the good jumps, from tmp file if necessary
 * writejumps() -- write a filled array of jumps to a tmp file: nw()
 */
10 #include "nw.h"
#include <sys/file.h>

char    *jname = "/tmp/homgXXXXXX";          /* tmp file for jumps */
FILE    *fj;

15 int     cleanup();                          /* cleanup tmp file */
long    lseek();

/*
20 * remove any tmp file if we blow
 */
cleanup(i)                                cleanup
    int    i;
{
25     if (fj)
        (void) unlink(jname);
        exit(i);
}

30 /*
 * read, return ptr to seq, set dna, len, maxlen
 * skip lines starting with ';', '<', or '>'
 * seq in upper or lower case
 */
35 char    *
getseq(file, len)
    char    *file;    /* file name */
    int     *len;     /* seq len */
40 {
    char    line[1024], *pseq;
    register char    *px, *py;
    int     natgc, tlen;
    FILE    *fp;
45     if ((fp = fopen(file, "r")) == 0) {
        fprintf(stderr, "%s: can't read %s\n", prog, file);
        exit(1);
    }
50     tlen = natgc = 0;
    while (fgets(line, 1024, fp)) {
        if (*line == ';' || *line == '<' || *line == '>')
            continue;
        for (px = line; *px != '\n'; px++)
55             if (isupper(*px) || islower(*px))
                tlen++;
    }
    if ((pseq = malloc((unsigned)(tlen+6))) == 0) {
        fprintf(stderr, "%s: malloc() failed to get %d bytes for %s\n", prog, tlen+6, file);
60         exit(1);
    }
    pseq[0] = pseq[1] = pseq[2] = pseq[3] = '\0';

```

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Table 1 (cont')

```

5      py = pseq + 4;
      *len = tlen;
      rewind(fp);

      while (fgets(line, 1024, fp)) {
          if (*line == ';' || *line == '<' || *line == '>')
              continue;
10         for (px = line; *px != '\n'; px++) {
              if (isupper(*px))
                  *py++ = *px;
              else if (islower(*px))
                  *py++ = toupper(*px);
15         if (index("ATGCU", *(py-1)))
            natgc++;
        }
    }
    *py++ = '\0';
    *py = '\0';
    (void) fclose(fp);
    dna = natgc > (tlen/3);
    return(pseq+4);
25 }

char *
g_calloc(msg, nx, sz)
    char *msg;          /* program, calling routine */
    int nx, sz;          /* number and size of elements */
30 {
    char *px, *calloc();

    if ((px = calloc((unsigned)nx, (unsigned)sz)) == 0) {
        if (*msg) {
35             fprintf(stderr, "%s: g_calloc() failed %s (n=%d, sz=%d)\n", prog, msg, nx, sz);
            exit(1);
        }
    }
    return(px);
40 }

/*
 * get final jmps from dx[] or tmp file, set pp[], reset dmax: main()
 */
45 readjmps()
{
    readjmps
    {
        int fd = -1;
        int siz, i0, i1;
50     register i, j, xx;

        if (fj) {
            (void) fclose(fj);
            if ((fd = open(jname, O_RDONLY, 0)) < 0) {
55                 fprintf(stderr, "%s: can't open() %s\n", prog, jname);
                cleanup(1);
            }
        }
        for (i = i0 = i1 = 0, dmax0 = dmax, xx = len0; ; i++) {
60             while (1) {
                for (j = dx[dmax].ijmp; j >= 0 && dx[dmax].jp.x[j] >= xx; j--)
                    ;
            }
        }
    }
}

```

Table 1 (cont')

...readjumps

```

5      if (j < 0 && dx[dmax].offset && fj) {
        (void) lseek(fd, dx[dmax].offset, 0);
        (void) read(fd, (char *)&dx[dmax].jp, sizeof(struct jmp));
        (void) read(fd, (char *)&dx[dmax].offset, sizeof(dx[dmax].offset));
        dx[dmax].ijmp = MAXJMP-1;
      }
10     else
        break;
    }
    if (i >= JMPS) {
        fprintf(stderr, "%s: too many gaps in alignment\n", prog);
        cleanup(1);
15    }
    if (j >= 0) {
        siz = dx[dmax].jp.n[j];
        xx = dx[dmax].jp.x[j];
        dmax += siz;
20        if (siz < 0) { /* gap in second seq */
            pp[1].n[i1] = -siz;
            xx += siz;
            /* id = xx - yy + len1 - 1
            */
25            pp[1].x[i1] = xx - dmax + len1 - 1;
            gapy++;
            ngapy -= siz;
        /* ignore MAXGAP when doing endgaps */
            siz = (-siz < MAXGAP || endgaps)? -siz : MAXGAP;
30            i1++;
        }
        else if (siz > 0) { /* gap in first seq */
            pp[0].n[i0] = siz;
            pp[0].x[i0] = xx;
35            gapx++;
            ngapx += siz;
        /* ignore MAXGAP when doing endgaps */
            siz = (siz < MAXGAP || endgaps)? siz : MAXGAP;
40            i0++;
        }
    }
    else
        break;
}
45 /* reverse the order of jumps
*/
for (j = 0, i0--; j < i0; j++, i0--) {
    i = pp[0].n[j]; pp[0].n[j] = pp[0].n[i0]; pp[0].n[i0] = i;
    i = pp[0].x[j]; pp[0].x[j] = pp[0].x[i0]; pp[0].x[i0] = i;
50 }
for (j = 0, i1--; j < i1; j++, i1--) {
    i = pp[1].n[j]; pp[1].n[j] = pp[1].n[i1]; pp[1].n[i1] = i;
    i = pp[1].x[j]; pp[1].x[j] = pp[1].x[i1]; pp[1].x[i1] = i;
55 }
if (fd >= 0)
    (void) close(fd);
if (fj) {
    (void) unlink(jname);
    fj = 0;
60    offset = 0;
}
}

```

Table 1 (cont')

```

5  /*
   * write a filled jmp struct offset of the prev one (if any): nw()
   */
writejumps(ix)      writejumps
      int            ix;
{
10      char          *mktemp();

      if (!fj) {
15          if (mktemp(jname) < 0) {
              fprintf(stderr, "%s: can't mktemp() %s\n", prog, jname);
              cleanup(1);
          }
          if ((fj = fopen(jname, "w")) == 0) {
              fprintf(stderr, "%s: can't write %s\n", prog, jname);
              exit(1);
20          }
      }
      (void) fwrite((char *)&dx[ix].jp, sizeof(struct jmp), 1, fj);
      (void) fwrite((char *)&dx[ix].offset, sizeof(dx[ix].offset), 1, fj);
}

```

25

30

35

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60

65

Table 2

PRO	XXXXXXXXXXXXXX	(Length = 15 amino acids)
Comparison Protein	XXXXXXXXXXXX	(Length = 12 amino acids)

5

% amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =

10

5 divided by 15 = 33.3%

15

20

Table 3

PRO	XXXXXXXXXX	(Length = 10 amino acids)
Comparison Protein	XXXXXXXXXXXXZZ	(Length = 15 amino acids)

25

% amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =

30

5 divided by 10 = 50%

Table 4

PRO-DNA	NNNNNNNNNNNNNN	(Length = 14 nucleotides)
Comparison DNA	NNNNNNLLLLLLLL	(Length = 16 nucleotides)

% nucleic acid sequence identity =

(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2)
divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =

6 divided by 14 = 42.9%

Table 5

PRO-DNA	NNNNNNNNNNNN	(Length = 12 nucleotides)
Comparison DNA	NNNNLLLV	(Length = 9 nucleotides)

% nucleic acid sequence identity =

(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2)
divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =

4 divided by 12 = 33.3%

III. Compositions and Methods of the Invention

A. Full-Length PRO polypeptides

5 The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO polypeptides. In particular, cDNAs encoding various PRO polypeptides have been identified and isolated, as disclosed in further detail in the Examples below. It is noted that proteins produced in separate expression rounds may be given different PRO numbers but the UNQ number is unique for any given DNA and the encoded protein, and will not be changed. However, for sake of simplicity, in the present specification the protein encoded by the full length
10 native nucleic acid molecules disclosed herein as well as all further native homologues and variants included in the foregoing definition of PRO, will be referred to as "PRO/number", regardless of their origin or mode of preparation.

As disclosed in the Examples below, various cDNA clones have been deposited with the ATCC. The actual nucleotide sequences of those clones can readily be determined by the skilled artisan by
15 sequencing of the deposited clone using routine methods in the art. The predicted amino acid sequence can be determined from the nucleotide sequence using routine skill. For the PRO polypeptides and encoding nucleic acids described herein, Applicants have identified what is believed to be the reading frame best identifiable with the sequence information available at the time.

20 B. PRO Polypeptide Variants

In addition to the full-length native sequence PRO polypeptides described herein, it is contemplated that PRO variants can be prepared. PRO variants can be prepared by introducing appropriate nucleotide changes into the PRO DNA, and/or by synthesis of the desired PRO polypeptide. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of the PRO, such as
25 changing the number or position of glycosylation sites or altering the membrane anchoring characteristics.

Variations in the native full-length sequence PRO or in various domains of the PRO described herein, can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Patent No. 5,364,934. Variations may be a substitution, deletion or insertion of one or more codons encoding the PRO that results in a change in the
30 amino acid sequence of the PRO as compared with the native sequence PRO. Optionally the variation is by substitution of at least one amino acid with any other amino acid in one or more of the domains of the PRO. Guidance in determining which amino acid residue may be inserted, substituted or deleted without adversely affecting the desired activity may be found by comparing the sequence of the PRO with that of homologous known protein molecules and minimizing the number of amino acid sequence changes made in regions of
35 high homology. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, *i.e.*, conservative amino acid replacements. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. The variation allowed may be determined by systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the
40 full-length or mature native sequence.

PRO polypeptide fragments are provided herein. Such fragments may be truncated at the N-terminus or C-terminus, or may lack internal residues, for example, when compared with a full length native protein. Certain fragments lack amino acid residues that are not essential for a desired biological activity of the PRO polypeptide.

5 PRO fragments may be prepared by any of a number of conventional techniques. Desired peptide fragments may be chemically synthesized. An alternative approach involves generating PRO fragments by enzymatic digestion, *e.g.*, by treating the protein with an enzyme known to cleave proteins at sites defined by particular amino acid residues, or by digesting the DNA with suitable restriction enzymes and isolating the desired fragment. Yet another suitable technique involves isolating and amplifying a DNA fragment
10 encoding a desired polypeptide fragment, by polymerase chain reaction (PCR). Oligonucleotides that define the desired termini of the DNA fragment are employed at the 5' and 3' primers in the PCR. Preferably, PRO polypeptide fragments share at least one biological and/or immunological activity with the native PRO polypeptide disclosed herein.

15 In particular embodiments, conservative substitutions of interest are shown in Table 6 under the heading of preferred substitutions. If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 6, or as further described below in reference to amino acid classes, are introduced and the products screened.

Table 6

	Original Residue	Exemplary Substitutions	Preferred Substitutions
20	Ala (A)	val; leu; ile	val
	Arg (R)	lys; gln; asn	lys
	Asn (N)	gln; his; lys; arg	gln
25	Asp (D)	glu	glu
	Cys (C)	ser	ser
	Gln (Q)	asn	asn
	Glu (E)	asp	asp
	Gly (G)	pro; ala	ala
30	His (H)	asn; gln; lys; arg	arg
	Ile (I)	leu; val; met; ala; phe; norleucine	leu
	Leu (L)	norleucine; ile; val; met; ala; phe	ile
35	Lys (K)	arg; gln; asn	arg
	Met (M)	leu; phe; ile	leu
	Phe (F)	leu; val; ile; ala; tyr	leu
	Pro (P)	ala	ala
	Ser (S)	thr	thr
40	Thr (T)	ser	ser
	Trp (W)	tyr; phe	tyr

Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

Substantial modifications in function or immunological identity of the PRO polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

- (1) hydrophobic: norleucine, met, ala, val, leu, ile;
- (2) neutral hydrophilic: cys, ser, thr;
- (3) acidic: asp, glu;
- (4) basic: asn, gln, his, lys, arg;
- (5) residues that influence chain orientation: gly, pro; and
- (6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, more preferably, into the remaining (non-conserved) sites.

The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter *et al.*, *Nucl. Acids Res.*, **13**:4331 (1986); Zoller *et al.*, *Nucl. Acids Res.*, **10**:6487 (1987)], cassette mutagenesis [Wells *et al.*, *Gene*, **34**:315 (1985)], restriction selection mutagenesis [Wells *et al.*, *Philos. Trans. R. Soc. London SerA*, **317**:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the PRO variant DNA.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant [Cunningham and Wells, *Science*, **244**: 1081-1085 (1989)]. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions [Creighton, *The Proteins*, (W.H. Freeman & Co., N.Y.); Chothia, *J. Mol. Biol.*, **150**:1 (1976)]. If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

C. Modifications of PRO

Covalent modifications of PRO are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of a PRO polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C- terminal residues of the PRO. Derivatization with bifunctional agents is useful, for instance, for crosslinking PRO to a water-insoluble support matrix or surface for use in the method for purifying anti-PRO antibodies, and vice-versa.

Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[(p-azidophenyl)dithio]propioimide.

Other modifications include deamidation of glutamyl and asparaginy residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α -amino groups of lysine, arginine, and histidine side chains [T.E. Creighton, *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)], acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the PRO polypeptide included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in native sequence PRO (either by removing the underlying glycosylation site or by deleting the glycosylation by chemical and/or enzymatic means), and/or adding one or more glycosylation sites that are not present in the native sequence PRO. In addition, the phrase includes qualitative changes in the glycosylation of the native proteins, involving a change in the nature and proportions of the various carbohydrate moieties present.

Addition of glycosylation sites to the PRO polypeptide may be accomplished by altering the amino acid sequence. The alteration may be made, for example, by the addition of, or substitution by, one or more serine or threonine residues to the native sequence PRO (for O-linked glycosylation sites). The PRO amino acid sequence may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the PRO polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

Another means of increasing the number of carbohydrate moieties on the PRO polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Such methods are described in the art, e.g., in WO 87/05330 published 11 September 1987, and in Aplin and Wriston, *CRC Crit. Rev. Biochem.*, pp. 259-306 (1981).

Removal of carbohydrate moieties present on the PRO polypeptide may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. Chemical deglycosylation techniques are known in the art and described, for instance, by Hakimuddin, *et al.*, *Arch. Biochem. Biophys.*, 259:52 (1987) and by Edge *et al.*, *Anal. Biochem.*, 118:131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.*, *Meth. Enzymol.*, 138:350 (1987).

Another type of covalent modification of PRO comprises linking the PRO polypeptide to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol (PEG), polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

The PRO of the present invention may also be modified in a way to form a chimeric molecule comprising PRO fused to another, heterologous polypeptide or amino acid sequence.

In one embodiment, such a chimeric molecule comprises a fusion of the PRO with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl- terminus of the PRO. The presence of such epitope-tagged forms of the PRO can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the PRO to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field *et al.*, *Mol. Cell. Biol.*, 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan *et al.*, *Molecular and Cellular Biology*, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky *et al.*, *Protein Engineering*, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag-peptide [Hopp *et al.*, *BioTechnology*, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin *et al.*, *Science*, 255:192-194 (1992)]; an α -tubulin epitope peptide [Skinner *et al.*, *J. Biol. Chem.*, 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:6393-6397 (1990)].

In an alternative embodiment, the chimeric molecule may comprise a fusion of the PRO with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule (also referred to as an "immunoadhesin"), such a fusion could be to the Fc region of an IgG molecule. The Ig fusions preferably include the substitution of a soluble (transmembrane domain deleted or inactivated) form of a PRO polypeptide in place of at least one variable region within an Ig molecule. In a particularly preferred embodiment, the immunoglobulin fusion includes the hinge, CH2 and CH3, or the hinge, CH1, CH2 and CH3 regions of an IgG1 molecule. For the production of immunoglobulin fusions see also US Patent No. 5,428,130 issued June 27, 1995.

D. Preparation of PRO

The description below relates primarily to production of PRO by culturing cells transformed or transfected with a vector containing PRO nucleic acid. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare PRO. For instance, the PRO sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques [see, e.g., Stewart *et al.*, *Solid-Phase Peptide Synthesis*, W.H. Freeman Co., San Francisco, CA (1969); Merrifield, *J. Am. Chem. Soc.*, 85:2149-2154 (1963)]. *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of the PRO may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the full-length PRO.

1. Isolation of DNA Encoding PRO

DNA encoding PRO may be obtained from a cDNA library prepared from tissue believed to possess the PRO mRNA and to express it at a detectable level. Accordingly, human PRO DNA can be conveniently obtained from a cDNA library prepared from human tissue, such as described in the Examples. The PRO-encoding gene may also be obtained from a genomic library or by known synthetic procedures (e.g., automated nucleic acid synthesis).

Libraries can be screened with probes (such as antibodies to the PRO or oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the gene encoding PRO is to use PCR methodology [Sambrook *et al.*, *supra*; Dieffenbach *et al.*, *PCR Primer: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1995)].

The Examples below describe techniques for screening a cDNA library. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The oligonucleotide is preferably labeled such that it can be detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like ³²P-labeled ATP, biotinylation or enzyme labeling. Hybridization conditions, including moderate stringency and high stringency, are provided in Sambrook *et al.*, *supra*.

Sequences identified in such library screening methods can be compared and aligned to other known sequences deposited and available in public databases such as GenBank or other private sequence databases. Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined using methods known in the art and as described herein.

Nucleic acid having protein coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in Sambrook *et al.*, *supra*, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

2. Selection and Transformation of Host Cells

Host cells are transfected or transformed with expression or cloning vectors described herein for PRO production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in *Mammalian Cell Biotechnology: A Practical Approach*, M. Butler, ed. (IRL Press, 1991) and Sambrook *et al.*, *supra*.

Methods of eukaryotic cell transfection and prokaryotic cell transformation are known to the ordinarily skilled artisan, for example, CaCl₂, CaPO₄, liposome-mediated and electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells. The

calcium treatment employing calcium chloride, as described in Sambrook *et al.*, *supra*, or electroporation is generally used for prokaryotes. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, *Gene*, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, *Virology*, 52:456-457 (1978) can be employed. General aspects of mammalian cell host system transfections have been described in U.S. Patent No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, *J. Bact.*, 130:946 (1977) and Hsiao *et al.*, *Proc. Natl. Acad. Sci. (USA)*, 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, e.g., polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown *et al.*, *Methods in Enzymology*, 185:527-537 (1990) and Mansour *et al.*, *Nature*, 336:348-352 (1988).

Suitable host cells for cloning or expressing the DNA in the vectors herein include prokaryote, yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *E. coli*. Various *E. coli* strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635). Other suitable prokaryotic host cells include Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (e.g., *B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. These examples are illustrative rather than limiting. Strain W3110 is one particularly preferred host or parent host because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding proteins endogenous to the host, with examples of such hosts including *E. coli* W3110 strain 1A2, which has the complete genotype *tonA*; *E. coli* W3110 strain 9E4, which has the complete genotype *tonA ptr3*; *E. coli* W3110 strain 27C7 (ATCC 55,244), which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT kan'*; *E. coli* W3110 strain 37D6, which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT rbs7 ilvG kan'*; *E. coli* W3110 strain 40B4, which is strain 37D6 with a non-kanamycin resistant *degP* deletion mutation; and an *E. coli* strain having mutant periplasmic protease disclosed in U.S. Patent No. 4,946,783 issued 7 August 1990. Alternatively, *in vitro* methods of cloning, e.g., PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for PRO-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe* (Beach and Nurse, *Nature*, 290: 140 [1981]; EP 139,383 published 2 May 1985); *Kluyveromyces* hosts (U.S. Patent No. 4,943,529; Fleer *et al.*, *Bio/Technology*, 9:968-975 (1991)) such as, e.g., *K. lactis* (MW98-8C, CBS683, CBS4574; Louvencourt *et al.*, *J. Bacteriol.*, 154(2):737-742 [1983]), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilum* (ATCC 36,906; Van den Berg *et al.*, *Bio/Technology*, 8:135 (1990)), *K. thermotolerans*, and *K. marxianus*;

yarrowia (EP 402,226); *Pichia pastoris* (EP 183,070; Sreekrishna *et al.*, *J. Basic Microbiol.*, 28:265-278 [1988]); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa* (Case *et al.*, *Proc. Natl. Acad. Sci. USA*, 76:5259-5263 [1979]); *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 October 1990); and filamentous fungi such as, *e.g.*, *Neurospora*, *Penicillium*, *Tolypocladium* (WO 91/00357 published 10 January 1991), and *Aspergillus* hosts such as *A. nidulans* (Ballance *et al.*, *Biochem. Biophys. Res. Commun.*, 112:284-289 [1983]; Tilburn *et al.*, *Gene*, 26:205-221 [1983]; Yelton *et al.*, *Proc. Natl. Acad. Sci. USA*, 81: 1470-1474 [1984]) and *A. niger* (Kelly and Hynes, *EMBO J.*, 4:475-479 [1985]). Methylotropic yeasts are suitable herein and include, but are not limited to, yeast capable of growth on methanol selected from the genera consisting of *Hansenula*, *Candida*, *Kloeckera*, *Pichia*, *Saccharomyces*, *Torulopsis*, and *Rhodotorula*. A list of specific species that are exemplary of this class of yeasts may be found in C. Anthony, *The Biochemistry of Methylotrophs*, 269 (1982).

Suitable host cells for the expression of glycosylated PRO are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as *Drosophila* S2 and *Spodoptera* Sf9, as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen Virol.*, 36:59 (1977)); Chinese hamster ovary cells/DHFR (CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.*, 23:243-251 (1980)); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor (MMT 060562, ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

3. Selection and Use of a Replicable Vector

The nucleic acid (*e.g.*, cDNA or genomic DNA) encoding PRO may be inserted into a replicable vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally include, but are not limited to, one or more of a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

The PRO may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the PRO-encoding DNA that is inserted into the vector. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, *e.g.*, the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* α -factor leaders, the latter described in U.S. Patent No. 5,010,182), or acid phosphatase leader, the *C. albicans*

glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders.

5 Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells.

10 Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*.

An example of suitable selectable markers for mammalian cells are those that enable the
15 identification of cells competent to take up the PRO-encoding nucleic acid, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described by Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980). A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 [Stinchcomb *et al.*, *Nature*, 282:39 (1979); Kingsman *et al.*, *Gene*, 7:141 (1979); Tschemper *et al.*, *Gene*, 10:157 (1980)].
20 The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 [Jones, *Genetics*, 85:12 (1977)].

Expression and cloning vectors usually contain a promoter operably linked to the PRO-encoding nucleic acid sequence to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known. Promoters suitable for use with prokaryotic hosts include the β -lactamase and lactose
25 promoter systems [Chang *et al.*, *Nature*, 275:615 (1978); Goeddel *et al.*, *Nature*, 281:544 (1979)], alkaline phosphatase, a tryptophan (*trp*) promoter system [Goeddel, *Nucleic Acids Res.*, 8:4057 (1980); EP 36,776], and hybrid promoters such as the *tac* promoter [deBoer *et al.*, *Proc. Natl. Acad. Sci. USA*, 80:21-25 (1983)]. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding PRO.

30 Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman *et al.*, *J. Biol. Chem.*, 255:2073 (1980)] or other glycolytic enzymes [Hess *et al.*, *J. Adv. Enzyme Reg.*, 7:149 (1968); Holland, *Biochemistry*, 17:4900 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase,
35 phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and

galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657.

PRO transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible with the host cell systems.

Transcription of a DNA encoding the PRO by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the PRO coding sequence, but is preferably located at a site 5' from the promoter.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding PRO.

Still other methods, vectors, and host cells suitable for adaptation to the synthesis of PRO in recombinant vertebrate cell culture are described in Gething *et al.*, *Nature*, 293:620-625 (1981); Mantei *et al.*, *Nature*, 281:40-46 (1979); EP 117,060; and EP 117,058.

4. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA [Thomas, *Proc. Natl. Acad. Sci. USA*, 77:5201-5205 (1980)], dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence PRO polypeptide or against a

synthetic peptide based on the DNA sequences provided herein or against exogenous sequence fused to PRO DNA and encoding a specific antibody epitope.

5. Purification of Polypeptide

Forms of PRO may be recovered from culture medium or from host cell lysates. If membrane-bound, it can be released from the membrane using a suitable detergent solution (e.g. Triton-X® 100) or by enzymatic cleavage. Cells employed in expression of PRO can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

It may be desired to purify PRO from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the PRO. Various methods of protein purification may be employed and such methods are known in the art and described for example in Deutscher, *Methods in Enzymology*, 182 (1990); Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag, New York (1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular PRO produced.

Many heterogeneous proteins expressed in *E. coli* require refolding in order to impart activity. When this is necessary, the following procedure can be used. For a general discussion of procedures suitable for refolding of recombinant or synthetic PRO polypeptide including any N- or C-terminal extended forms, the reader is referred to the following patents: Builder *et al.*, U.S. Patent No. 4,511,502; Jones *et al.*, U.S. Patent 4,512,922; Olson, U.S. Patent No. 4,518,526; Builder *et al.*, U.S. Patent 4,620,948.

IV. Therapeutic Indications

Various ocular disorders can result in the death of retinal cells. These include such widely disparate conditions as detachment of the retina and pigment epithelium, degenerative myopia, acute retinal necrosis syndrome (ARN), and traumatic chorioretinopathies or contusion (Purtscher's Retinopathy). Retinal tears is a condition characterized by where the retina tears or separates from the underlying choroid, which can sometimes result in rupturing of the choroid. Retinal tears may occur for a wide variety of reasons. Particularly troublesome are macular holes which produce blurred central vision or metamorphopsia.

While the direct cause of most macular holes is unknown, they have been associated with trauma, cystic degeneration and vitreoretinal traction. Also, full thickness macular holes have appeared following myopic degeneration, laser photocoagulation, lightning strike and pilocarpine administration. Macular holes are also present in high frequency after cataract extraction. A particular form of acute macular holes is idiopathic senile macular hole, which involves a full thickness hole through the macula surrounded by annular retinal detachment. It is believed that macular holes begin with central or foveolar detachment, which then eventually develops into a full-depth macular hole. Gass *et al.*, *Arch. Ophthalmol.* 106: 629-639 (1988). While surgical procedures, such as trans-*para plana* vitrectomy may interrupt the progress of

macular degeneration to a full blown macular hole, this operation can permanently damage central vision, and typically only improves vision 40% of the time.

5 Other ocular disorders which can result in photoreceptor cell death include edema, ischemic conditions and uveitis. Macular and retinal edema are often associated with metabolic illnesses such as diabetes mellitus. Retinal edema is found in a large percentage of individuals who have undergone cataract extraction and other surgical procedures upon the eye. Edema is also found with accelerated or malignant hypertension. Macular edema is a common complication of prolonged inflammation due to uveitis, Eales disease, or other diseases. Local edema is associated with multiple cystoid bodies ("cotton bodies") as a result of AIDS.

10 Retinal ischemia can occur from either choroidal or retinal vascular diseases, such as central or branch retinal vision occlusion, collagen vascular diseases and thrombocytopenic purpura. Retinal vasculitis and occlusion is seen with Eales disease and systemic lupus erythematosus.

Age-related macular degeneration (AMD) is the major cause of severe visual loss in United States citizens over the age of 55. AMD may occur either in an atrophic or exudative form. Most AMD patients have a build up of deposits within and under the retinal pigment epithelium in the macular region resulting in atrophy of the retina and the retinal pigment epithelium. The retinal pigment scavenge for photoreceptor discs from the rods and cones for years and accumulate intracellular wastes. The incompletely digested residues reduce cytoplasmic space and interfere with metabolism. Feeny-Burns, *et al.*, *Invest Ophthalmol. Mol. Vis. Sci.* (1984), 25: 195-200. As the cell volume available to the organelles diminishes, the capacity to digest photoreceptors decreases, which may be the basis for macular degeneration.

Exudative AMD is characterized by the growth of blood vessels from the choriocapillaris through defects in Bruch's membrane, and in some cases the underlying retinal pigment epithelium (RPE). The accumulation of serous or hemorrhagic exudates escaping from these vessels results in fibrous scarring of the macular region with attendant degeneration of the neuroretina and permanent loss of central vision.

25 Exudative AMD has also been associated with choroidal neovascularization, detachment and tears of the retinal pigment epithelium. The cascade retinal events is responsible for more than 80% of cases of significant visual loss in patients with AMD.

Laser photocoagulation has been attempted in an effort to ameliorate the initial or recurrent neovascular lesions associated with AMD. *Arch. Ophthalmol.* (1991) 109: 1220; *Arch. Ophthalmol.* (1991) 109: 1232; *Arch. Ophthalmol.* (1991) 109:1242. Unfortunately, AMD patients with subfoveal lesions subjected to laser treatment experienced a severe reduction in visual acuity (mean 3 lines) at 3 months follow-up. Moreover, at two years post-treatment treated eyes had only marginally better visual acuity than their untreated counterparts (means of 20/320 and 20/400, respectively). Another drawback of the procedure is that vision immediately after surgery is worse.

35 As a result, the retinal neuron survival agents of the present invention are promising candidates for the treatment of retinal detachment, retinal tears, degenerative myopia, acute retinal necrosis syndrome (ARN), and traumatic chorioretinopathies or contusion (including Purtscher's retinopathy), macular holes (including idiopathic senile macular holes), macular and retinal edema, macular degeneration (including age-

related macular degeneration or AMD), edema, ischemic conditions (e.g., central or branch retinal vision occlusion, collagen vacuolar diseases, thrombocytopenic purpura), uveitis and retinal vasculitis and occlusion associated with Eales disease and systemic lupus erythematosus.

5 **V. Modes For Carrying Out The Invention:**

A. Retinal Neuron (including photoreceptor) Survival assays:

 In these assays, neural retinas are removed from pigment epithelium and dissociated into a single cell suspension using 0.25% trypsin in Ca^{2+} , Mg^{2+} -free PBS. The cells are then plated out in 96-well plates at 100,000 cells per well in DMEM/F12 supplemented with N2. After 2-3 days in culture, the cells are fixed
10 and stained. Since death typically occurs upon detachment of neural retinal cells from the underlying pigment epithelium the relative survival enhancing effect of the tested agent can be readily determined by comparison with the untreated control wells.

 The procedure is described in greater detail in the examples.

15 **B. Age-related macular degeneration (AMD):**

 In this assay, the effectiveness and safety of locally administered PRO polypeptide can be examined using a procedure substantially similar to that outlined in WO 94/01124, filed 8 July 1993 which describes subretinal or intravitreal injections of retinal survival promoting therapeutic agents. Briefly, patients with visual acuity of 20/160 or better with a recent diagnosis of AMD are examined for change in
20 visual acuity from baseline and stabilization. Study parameters should measure best corrected visual acuity for both distance and near vision, intraocular pressure, lens status and refraction. The amount of serous and hyperfluorescence from classic/occult neovascularization, total lesion size and foveal involvement are also measured on fluorescein angiography and ICG (indocyanine green) angiography.

25 **C. Macular holes:**

 In this assay, the safety and effectiveness of locally administered PRO polypeptide can be examined using a procedure substantially similar to that outlined in WO 94/01124, filed 8 July 1993 which describes subretinal or intravitreal injection of retinal survival promoting therapeutic agents. Briefly, patients with confirmed macular holes are examined for visual acuity and analyzed by intraocular pressure,
30 fundus photographs, and fluorescein angiography.

 The rationale for treatment is to induce the flattening of the edges of the macular holes in order to resolve retinal detachment and thickening surrounding the hole. It is believed that a reduction in the traction force which elevates the retina around the hole coupled with an induction of the chorioretinal adhesion along the edge of the hole is necessary for therapeutic effect. The procedure is described in more detail in
35 the examples.

D. Light-induced photoreceptor injury:

In this assay, an albino rat is maintained in first a cyclic light environment followed by exposure to a constant light source with and without administration of the tested photoreceptor survival agent. The intravitreal administration of factors into the eyes of albino rats enables the assessment of both the ability of the factors to rescue photoreceptors from degeneration as well as the side effects, such as incidence of macrophages, associated with each factor.

Briefly, rats are given intraocular injections prior to constant light exposure and compared to control animals who received sham and no injections. Subsequent to constant light exposure, the eyes are removed, embedded in epoxy resin and sectioned along the vertical meridian. The degree of light-induced retinal degeneration can be measured first by examining the outer nuclear layer thickness and second by a subjective score assigned to the relative integrity of the retina.

E. Light ablation:

In this assay, the degree of photoreceptor rescue is measured in female Sprague-Dawley rats in a modification of the procedure described in Reme *et al.*, *Degen. Dis. Retina*, Ch. 3, Ed. R.E. Anderson *et al.*, Plenum Press, New York (1995). Briefly, animals are first acclimated to cyclical lighting, followed by immersion in total darkness. Animals are injected with test factor prior to intermittent light exposure. The degree of retinal degeneration or survival promoting activity of the tested factors is reported as the thickness of the photoreceptor cell layer or number of TUNEL labeled photoreceptor cell nuclei.

F. Corneal Pocket assay:

In this assay, particular agents are tested to determine whether they are angiogenic under a procedure adapted from Polverini *et al.*, *Methods Enzymol.* 198: 440-450 (1991). Briefly, Sprague-Dawley are anesthetized, secured and an incision is made in their corneas into which is placed a pellet of the test factor in combination with sucralfate and Hydron.

G. Vascular Endothelial Cell Mitogenicity Assay:

This particular assay measures the mitogenicity (*e.g.*, angiogenesis) of the test factor on vascular endothelial cells. It was developed as a reliable means of measuring the purification of bFGF (SEQ ID NO:3) as described by Ferrara, *et al.*, *Methods of Enzymology* 198: 391-405 (1991). Briefly, bovine adrenal cortex-derived cells are grown and maintained in culture in the presence of low glucose DMEM, the test factor is administered and test cultures vs. controls are measured.

H. Administration Methods:

The PRO polypeptides of the present invention can be delivered to the eye through a variety of routes. Methods of introduction include any mode of administration known in the art, including but not limited to intravenously, intraarterially, intrathecally, subcutaneously, intradermally, by injection into involved tissue, intranasally, intramuscularly, intraperitoneally, orally, or via an implanted device. They may be delivered intraocularly, by topical application to the eye or by intraocular injection into, for example

the vitreous or subretinal (interphotoreceptor) space. Alternatively, they may be delivered locally by insertion or injection into the tissue surrounding the eye. They may be delivered systemically through an oral route or by subcutaneous, intravenous or intramuscular injection. Alternatively, they may be delivered by means of a catheter or by means of an implant, wherein such an implant is made of a porous, non-porous or gelatinous material, including membranes such as silastic membrane or fibers, biodegradable polymers, or proteinaceous material. All of the above delivery methods may also be carried out by means of a sustained release delivery system. The factors may be administered prior to the onset of the condition, to prevent its occurrence, for example, during surgery on the eye, or immediately after the onset of the pathological condition or during the occurrence of an acute or protracted condition.

Intravitreal injection of potential retinal neuron survival promoting factors has several advantages over systemic applications. The amount of any specific agent that reaches the retina can be more accurately determined, since the eye is a round, relatively contained structure and the agent is injected directly into it. Moreover, the amount of agent that needs to be injected is minuscule compared to systemic injections. For example, a single microliter in volume (about 1 microgram of agent) is used for intravitreal injection, as compared to one to several milliliters (ten to several hundred milligrams of agent) necessary for systemic injections. In addition, the intravitreal route of administration avoids the potentially toxic effect of some agents.

Further, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment, this may be achieved by, for example, local infusion during surgery, by injection, by means of a catheter, or by means of an implant, wherein such implant can be of a porous, non-porous, or gelatinous material, including membranes, such as silastic membranes or fibers.

The factors of the present invention may be modified to enhance their ability to penetrate the blood-retinal barrier. Such modification may include increasing their lipophilicity by, for example, glycosylation, or increasing their net charge by methods known in the art.

The factors may be delivered alone or in combination, and may be delivered along with a pharmaceutically acceptable vehicle. Ideally, such a vehicle would enhance the stability and/or delivery properties. The invention also provides for pharmaceutical compositions containing the active factor or fragment or derivative thereof, which can be administered using a suitable vehicle such as liposomes, microparticles or microcapsules. In various embodiments of the invention, it may be useful to use such compositions to achieve sustained release of the active component.

I. Pharmaceutical Compositions and Dosages

Therapeutic formulations of the polypeptide or antibody are prepared for storage as lyophilized formulations or aqueous solutions by mixing the polypeptide having the desired degree of purity with optional "pharmaceutically-acceptable" or "physiologically-acceptable" carriers, excipients or stabilizers typically employed in the art (all of which are termed "excipients"). For example, buffering agents, stabilizing agents, preservatives, isotonicifiers, non-ionic detergents, antioxidants and other miscellaneous additives. (See *Remington's Pharmaceutical Sciences*, 16th edition, A. Osol, Ed. (1980)). Such additives must be nontoxic to the recipients at the dosages and concentrations employed.

Buffering agents help to maintain the pH in the range which approximates physiological conditions. They are preferably present at concentration ranging from about 2mM to about 50 mM. Suitable buffering agents for use with the present invention include both organic and inorganic acids and salts thereof such as citrate buffers (*e.g.*, monosodium citrate-disodium citrate mixture, citric acid-trisodium citrate mixture, citric acid-monosodium citrate mixture, *etc.*), succinate buffers (*e.g.*, succinic acid-monosodium succinate mixture, succinic acid-sodium hydroxide mixture, succinic acid-disodium succinate mixture, *etc.*), tartrate buffers (*e.g.*, tartaric acid-sodium tartrate mixture, tartaric acid-potassium tartrate mixture, tartaric acid-sodium hydroxide mixture, *etc.*), fumarate buffers (*e.g.*, fumaric acid-monosodium fumarate mixture, *etc.*), fumarate buffers (*e.g.*, fumaric acid-monosodium fumarate mixture, fumaric acid-disodium fumarate mixture, monosodium fumarate-disodium fumarate mixture, *etc.*), gluconate buffers (*e.g.*, gluconic acid-sodium glyconate mixture, gluconic acid-sodium hydroxide mixture, gluconic acid-potassium glyconate mixture, *etc.*), oxalate buffer (*e.g.*, oxalic acid-sodium oxalate mixture, oxalic acid-sodium hydroxide mixture, oxalic acid-potassium oxalate mixture, *etc.*), lactate buffers (*e.g.*, lactic acid-sodium lactate mixture, lactic acid-sodium hydroxide mixture, lactic acid-potassium lactate mixture, *etc.*) and acetate buffers (*e.g.*, acetic acid-sodium acetate mixture, acetic acid-sodium hydroxide mixture, *etc.*). Additionally, there may be mentioned phosphate buffers, histidine buffers and trimethylamine salts such as Tris.

Preservatives are added to retard microbial growth, and are added in amounts ranging from 0.2% - 1% (w/v). Suitable preservatives for use with the present invention include phenol, benzyl alcohol, *meta*-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalconium halides (*e.g.*, chloride, bromide, iodide), hexamethonium chloride, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol.

Isotonicifiers sometimes known as "stabilizers" are present to ensure isotonicity of liquid compositions of the present invention and include polyhydric sugar alcohols, preferably trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol. Polyhydric alcohols can be present in an amount between 0.1% to 25% by weight, preferably 1% to 5% taking into account the relative amounts of the other ingredients.

Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which solubilizes the therapeutic agent or helps to prevent denaturation or adherence to the container wall. Typical stabilizers can be polyhydric sugar alcohols (enumerated above); amino acids such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, threonine, *etc.*, organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinositol, galactitol, glycerol and the like, including cyclitols such as inositol; polyethylene glycol; amino acid polymers; sulfur containing reducing agents, such as urea, glutathione, thiocetic acid, sodium thioglycolate, thioglycerol, α -monothioglycerol and sodium thio sulfate; low molecular weight polypeptides (*i.e.* < 10 residues); proteins such as human serum albumin, bovine serum albumin, gelatin or immunoglobulins; hydrophilic polymers, such as polyvinylpyrrolidone monosaccharides, such as xylose, mannose, fructose, glucose; disaccharides such as lactose, maltose, sucrose and trisaccharides such as raffinose; polysaccharides such as dextran. Stabilizers can be present in the range from 0.1 to 10,000 weights per part of weight active protein.

Non-ionic surfactants or detergents (also known as "wetting agents") are present to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stressed without causing denaturation of the protein. Suitable non-ionic surfactants include polysorbates (20, 80, *etc.*), polyoxamers (184, 188 *etc.*),
5 Pluronic® polyols, polyoxyethylene sorbitan monoethers (Tween®-20, Tween®-80, *etc.*). Non-ionic surfactants are present in a range of about 0.05 mg/ml to about 1.0 mg/ml, preferably about 0.07 mg/ml to about 0.2 mg/ml.

Additional miscellaneous excipients include bulking agents, (*e.g.* starch), chelating agents (*e.g.* EDTA), antioxidants (*e.g.*, ascorbic acid, methionine, vitamin E), and cosolvents.

10 The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide an immunosuppressive agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsule prepared, for example, by
15 coascervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsule and poly-(methylmethacrylate) microcapsule, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences*, 16th edition, A. Osal, Ed. (1980).

20 The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished, for example, by filtration through sterile filtration membranes.

Where sustained release administration of a PRO polypeptide is desired in a formulation with release characteristics suitable for the treatment of any disease or disorder requiring administration of the respective polypeptide, microencapsulation of the respective PRO polypeptide is contemplated.
25 Microencapsulation of recombinant proteins for sustained release had been successfully performed with human growth hormone (rhGH), interferon (rhIFN), interleukin-2, and MN rgp120. Johnson *et al.*, *Nat. Med.*, 2: 795-799 (1996); Yasuda, *Biomed. Ther.* 27: 1221-1223 (1993); Hora *et al.*, *Bio/Technology* 8: 755-758 (1990); Cleland, "Design and Production of Single Immunization Vaccines Using Polylactide Polyglycolide Microsphere Systems," in *Vaccine Design: The Subunit and Adjuvant Approach*, Powell and Newman, eds. (Plenum Press: New York, 1995), pp. 439-462; WO 97/03692, WO 96/40072, WO 96/07399; and U.S. Pat. No. 5,654,010.

Sustained-release formulations may be employed with the method of this invention, alternatively through the use of poly-lactic-coglycolic acid (PLGA) polymer due to its biocompatibility and wide range of biodegradable properties. The degradation products of PLGA, lactic and glycolic acids, can be cleared
35 quickly within the human body. Moreover, the degradability of this polymer can be adjusted from months to years depending on its molecular weight and composition. Lewis, "Controlled Release of Bioactive Agents from Lactide/Glycolide Polymer," in: M. Chasin and R. Langer (Eds.), *Biodegradable Polymers as Drug Delivery Systems* (Marcel Dekker: New York, 1990), pp. 1-41.

Alternatively still, suitable examples of sustained-release preparations include semi-permeable
40 matrices of solid hydrophobic polymers containing the antibody mutant, which matrices are in the form of

shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No.3,773,919), copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

The amount of therapeutic polypeptide, antibody or fragment thereof which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. Where possible, it is desirable to determine the dose-response curve and the pharmaceutical compositions of the invention first *in vitro*, and then in useful animal model systems prior to testing in humans. However, based on common knowledge of the art, a pharmaceutical composition effective in promoting the survival of sensory neurons may provide a local therapeutic agent concentration of between about 5 and 20 ng/ml, and, preferably, between about 10 and 20 ng/ml. In an additional specific embodiment of the invention, a pharmaceutical composition effective in promoting the growth and survival of retinal neurons may provide a local therapeutic agent concentration of between about 10 ng/ml and 100 ng/ml.

In a preferred embodiment, an aqueous solution of therapeutic polypeptide, antibody or fragment thereof is administered by subcutaneous injection. Each dose may range from about 0.5 µg to about 50 µg per kilogram of body weight, or more preferably, from about 3 µg to about 30 µg per kilogram body weight.

The dosing schedule for subcutaneous administration may vary from once a week to daily depending on a number of clinical factors, including the type of disease, severity of disease, and the subject's sensitivity to the therapeutic agent.

The amount of PRO protein which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. Where possible, it is desirable to determine the dose-response curve and the pharmaceutical compositions of the invention first *in vitro*, and then in useful animal model systems prior to testing in humans. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W., "The Use of Interspecies Scaling in Toxicokinetics", In Toxicokinetics and New Drug Development, Yacobi *et al.*, Eds. Pergamon Press, New York 1989, pp.42-96.

However, based on common knowledge of the art, a pharmaceutical composition effective in promoting the survival of sensory neurons may provide a local PRO protein concentration of between about

10 and 1000 ng/ml, preferably between 100 and 800 ng/ml and most preferably between about 200 ng/ml and 600 ng/ml of PRO polypeptide. Guidance as to particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Pat. Nos. 4,657,760; 5,206,344; or 5,224,212. It is contemplated that different formulations will be effective for different treatment compounds and different disorders, that administration targeting one organ or tissue, for example, may necessitate delivery in a manner different from that to another organ or tissue.

The dosing schedule for subvitreal administration of PRO may vary from once a week to daily depending on a number of clinical factors, including the type of disease, severity of disease, and the subject's sensitivity to PRO, respectively. Nonlimiting examples of dosing schedules are 3 µg/kg administered twice a week, three times a week or daily, a dose of 7 µg/kg twice a week, three times a week or daily, a dose of 10 µg/kg twice a week, three times a week or daily.

Effective doses of additional neurotrophic factors administered in combination with PRO polypeptide are in the same dose ranges as the effective dose of the principle respective active compound described herein. The active compound of the present method, PRO polypeptide may optionally be formulated with a second agent, such as a neurotrophic factor. Exemplary neurotrophic factors include: nerve growth factor (NGF), aGF, ciliary neurotrophic factor (CNTF), bovine-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), aFGF, IL-1β, TNFα, Insulin-like growth factor (IGF-1, IGF-2), transforming growth factor beta (TGF-β, TGF-β1) or skeletal muscle extract, may be administered in any sterile biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. However, certain factors such as bFGF, CNTF or IL-1β should be employed cautiously, as these agents may cause undesirable retinal complications such as macrophage proliferation, disorganization or the retinal structure, cell proliferation or inflammation.

If the subject manifests undesired side effects such as temperature elevation, cold or flu-like symptoms, fatigue, *etc.*, it may be desirable to administer a lower dose at more frequent intervals. One or more additional drugs may be administered in combination with PRO polypeptide to alleviate such undesired side effects, for example, an anti-pyretic, anti-inflammatory or analgesic agent.

J. PRO Agonists/Antagonists

This invention encompasses methods of screening compounds to identify those that mimic the PRO polypeptide (agonists) or prevent the effect of the respective polypeptide (antagonists). Screening assays for antagonist drug candidates are designed to identify compounds that bind or complex with the PRO polypeptides encoded by the genes identified herein, or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates.

The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays, and cell-based assays, which are well characterized in the art.

All assays for antagonists are common in that they call for contacting the drug candidate with a PRO polypeptide encoded by a nucleic acid identified herein under conditions and for a time sufficient to allow these two components to interact.

In binding assays, the interaction is binding and the complex formed can be isolated or detected in the reaction mixture. In a particular embodiment, the PRO polypeptide encoded by the gene identified herein or the drug candidate is immobilized on a solid phase, e.g., on a microtiter plate, by covalent or non-covalent attachments. Non-covalent attachment generally is accomplished by coating the solid surface with a solution of the PRO polypeptide and drying. Alternatively, an immobilized antibody, e.g., a monoclonal antibody, specific for the PRO polypeptide to be immobilized can be used to anchor it to a solid surface. The assay is performed by adding the non-immobilized component, which may be labeled by a detectable label, to the immobilized component, which may be labeled by a detectable label, to the immobilized component, e.g., the coated surface containing the anchored component. When the reaction is complete, the non-reacted components are removed, e.g., by washing, and complexes anchored on the solid surface are detected. When the originally non-immobilized component carries a detectable label, the detection of label immobilized on the surface indicates that complexing occurred. Where the originally non-immobilized component does not carry a label, complexing can be detected, for example, by using a labeled antibody specifically binding the immobilized complex.

If the candidate compound interacts with but does not bind to a particular PRO polypeptide encoded by a gene identified herein, its interaction with that polypeptide can be assayed by methods well known for detecting protein-protein interactions. Such assays include traditional approaches, such as, e.g., cross-linking, co-immunoprecipitation, and co-purification through gradients or chromatographic columns. In addition, protein-protein interactions can be monitored by using a yeast-based genetic system described by Fields and co-workers (Fields and Song, *Nature (London)* 340: 245-246 (1989); Chien *et al.*, *Proc. Natl. Acad. Sci. USA* 88: 9578-9582 (1991)) as disclosed by Chevray and Nathans, *Proc. Natl. Acad. Sci. USA* 89: 5789-5793 (1991). Many transcriptional activators, such as yeast GAL4, consist of two physically discrete modular domain, one acting as the DNA-binding domain, the other one functioning as the transcription-activation domain. The yeast expression system described in the foregoing publication (generally referred to as the "two-hybrid system") takes advantage of this property, and employs two hybrid proteins, one in which the target protein is fused to the DNA-binding domain of GAL4, and another, in which candidate activating proteins are fused to the activation domain. The expression of a GAL1-*lacZ* reporter gene under control of GAL4-activated promoter depends on reconstitution of GAL4 activity via protein-protein interaction. Colonies containing interacting polypeptides are detected with a chromogenic substrate for β -galactosidase. A complete kit (MATCHMAKER™) for identifying protein-protein interactions between two specific proteins using the two-hybrid technique is commercially available from Clontech. This system can also be extended to map protein domains involved in specific protein interactions as well as to pinpoint amino acid residues that are crucial for these interactions.

Compounds that interfere with the interaction of a gene encoding a PRO polypeptide identified herein and other intra- or extracellular components can be tested as follows: usually a reaction mixture is prepared containing the product of the gene and the intra- or extracellular component under conditions and for a time allowing for the interaction and binding of the two products. To test the ability of a candidate compound to inhibit binding, the reaction is run in the absence and in the presence of the test compound. In addition, a placebo may added to a third reaction mixture, to serve as a positive control. The binding (complex formation) between the test compound and the intra- or extracellular component present in the

mixture is monitored as described hereinabove. The formation of a complex in the control reaction(s) but not in the reaction mixture containing the test compound indicates that the test compound interferes with the interaction of the test compound and its reaction partner.

To assay for antagonists, the PRO polypeptide may be added to a cell along with the compound to be screened for a particular activity and the ability of the compound to inhibit the activity of interest in the presence of the respective PRO polypeptide indicates that the compound is an antagonist of the respective polypeptide. Alternatively, antagonists may be detected by combining the respective polypeptide and a potential antagonists with membrane-bound PRO polypeptide receptors or recombinant receptors under appropriate conditions for a competitive inhibition assay. The PRO polypeptide can be labeled, such as by radioactivity, such that the number of respective polypeptide molecules bound to the receptor can be used to determine the effectiveness of the potential antagonist. The gene encoding the receptor can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting. Coligan *et al.*, *Current Protocols in Immunol.* 1(2): Ch. 5 (1991). Preferably, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the PRO polypeptide and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to respective labeled polypeptide. The PRO polypeptide can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase. Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an interactive sub-pooling and re-screening process, eventually yielding a single clone that encodes the putative receptor.

In another assay for antagonists, mammalian cells or a membrane preparation expressing the receptor would be included with labeled PRO polypeptide, and, in particular, antibodies including, without limitation, poly- and monoclonal antibodies and antibody fragments, single-chain antibodies, anti-idiotypic antibodies, and chimeric or humanized versions of such antibodies or fragments, as well as human antibodies and antibody fragments. Alternatively, a potential antagonist may be a closely related protein, for example, a mutated form of the PRO polypeptide that recognizes the receptor but imparts no effect, thereby competitively inhibiting the action of the PRO polypeptide, respectively.

Another potential PRO polypeptide antagonist is an antisense RNA or DNA construct prepared using antisense technology, where, *e.g.*, an antisense RNA or DNA molecule acts to block directly the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, of which both methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5'-coding portion of the polynucleotide sequence, which encodes the mature PRO polypeptides herein, is used to design an antisense RNA oligonucleotide from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee *et al.*, *Nucl. Acids Res.* 6: 3073 (1979); Cooney *et al.*, *Science* 241: 456 (1988); Dervan *et al.*, *Science* 251: 1360 (1991)), thereby preventing transcription and the production of the PRO polypeptide. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into the PRO polypeptide (antisense - Okano, *Neurochem.* 56: 560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression* (CRC Press: Boca Raton, FL, 1988). The oligonucleotides

described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of the PRO polypeptide. When antisense DNA is used, oligodeoxylribonucleotides derived from the translation-initiation site, *e.g.*, between about -10 and +10 positions of the target gene sequence, are preferred.

5 Potential antagonists include small molecules that bind to the active site, the receptor binding site, or growth factor or other relevant binding site of the PRO polypeptide, thereby blocking the normal biological activity of the respective polypeptide. Examples of small molecules include, but are not limited to, small peptides or peptide-like molecules, preferably soluble peptides, and synthetic non-peptidyl organic or inorganic compounds.

10 Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. Ribozymes act by sequence-specific hybridization to the complementary target RNA, followed by endonucleolytic cleavage. Specific ribozyme cleavage sites within a potential RNA target can be identified by known techniques. For further details, see, *e.g.*, Rossi, *Current Biology* 4: 469-471 (1994), and PCT publication No. WO 97/33551 (published September 18, 1997).

15 Nucleic acid molecules in triple-helix formation used to inhibit transcription should be single-stranded and composed of deoxynucleotides. The base composition of these oligonucleotides is designed such that it promotes triple-helix formation via Hoogsteen base-pairing rules, which generally require sizeable stretches of purines of pyrimidines on one strand of a duplex. For further details see, *e.g.*, PCR publication No. WO 97/33551, *supra*.

20 These small molecules can be identified by any one of the screening assays discussed hereinabove and/or by any other screening techniques well known for those skilled in the art.

K. Anti-PRO Antibodies

25 The present invention further provides anti-PRO antibodies. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies.

1. Polyclonal Antibodies

30 The anti-PRO antibodies may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the PRO polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be
35 employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

2. Monoclonal Antibodies

The anti-PRO antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

The immunizing agent will typically include the PRO polypeptide or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, (1986) pp. 59-103]. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, Marcel Dekker, Inc., New York, (1987) pp. 51-63].

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against PRO. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, *Anal. Biochem.*, 107:220 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods [Goding, *supra*]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example,

protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; Morrison *et al.*, *supra*] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art.

3. Human and Humanized Antibodies

The anti-PRO antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region

(Fc), typically that of a human immunoglobulin [Jones *et al.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones *et al.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-327 (1988); Verhoeven *et al.*, *Science*, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991)]. The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies (Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985) and Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991)). Similarly, human antibodies can be made by introducing of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks *et al.*, *Bio/Technology* 10, 779-783 (1992); Lonberg *et al.*, *Nature* 368 856-859 (1994); Morrison, *Nature* 368, 812-13 (1994); Fishwild *et al.*, *Nature Biotechnology* 14, 845-51 (1996); Neuberger, *Nature Biotechnology* 14, 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13 65-93 (1995).

The antibodies may also be affinity matured using known selection and/or mutagenesis methods as described above. Preferred affinity matured antibodies have an affinity which is five times, more preferably 10 times, even more preferably 20 or 30 times greater than the starting antibody (generally murine, humanized or human) from which the matured antibody is prepared.

4. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the PRO, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities [Milstein and Cuello, *Nature*, 305:537-539 (1983)]. Because of the random assortment of immunoglobulin heavy and light chains, these

hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, *EMBO J.*, 10:3655-3659 (1991).

5 Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain
10 fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology*, 121:210 (1986).

 According to another approach described in WO 96/27011, the interface between a pair of
15 antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.* tyrosine or tryptophan). Compensatory
20 “cavities” of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.* alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

 Bispecific antibodies can be prepared as full length antibodies or antibody fragments (*e.g.* F(ab')₂, bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using
25 chemical linkage. Brennan *et al.*, *Science* 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')₂ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and
30 is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

 Fab' fragments may be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby *et al.*, *J. Exp. Med.* 175:217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')₂ molecule. Each Fab' fragment was separately secreted from *E. coli* and
35 subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

 Various technique for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced
40 using leucine zippers. Kostelny *et al.*, *J. Immunol.* 148(5):1547-1553 (1992). The leucine zipper peptides

from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber *et al.*, *J. Immunol.* 152:5368 (1994). Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al.*, *J. Immunol.* 147:60 (1991).

Exemplary bispecific antibodies may bind to two different epitopes on a given PRO polypeptide herein. Alternatively, an anti-PRO polypeptide arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (*e.g.* CD2, CD3, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular PRO polypeptide. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express a particular PRO polypeptide. These antibodies possess a PRO-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the PRO polypeptide and further binds tissue factor (TF).

5. Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells [U.S. Patent No. 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/200373; EP 03089]. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

6. Effector Function Engineering

It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, *e.g.*, the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) may be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, *J. Exp. Med.*, 176: 1191-1195 (1992) and Shopes, *J. Immunol.*, 148: 2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as

described in Wolff *et al. Cancer Research*, 53: 2560-2565 (1993). Alternatively, an antibody can be engineered that has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al., Anti-Cancer Drug Design*, 3: 219-230 (1989).

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7. Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ²¹²Bi, ¹³¹I, ¹³¹In, ⁹⁰Y, and ¹⁸⁶Re. Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis-(p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al., Science*, 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminedipentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

In another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.*, avidin) that is conjugated to a cytotoxic agent (*e.g.*, a radionucleotide).

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8. Immunoliposomes

The antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al., Proc. Natl. Acad. Sci. USA*, 82: 3688 (1985); Hwang *et al., Proc. Natl. Acad. Sci. USA*, 77: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be

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conjugated to the liposomes as described in Martin *et al.*, *J. Biol. Chem.*, 257: 286-288 (1982) via a disulfide-interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon *et al.*, *J. National Cancer Inst.*, 81(19): 1484 (1989).

9. Pharmaceutical Compositions of Antibodies

Antibodies specifically binding a PRO polypeptide identified herein, as well as other molecules identified by the screening assays disclosed hereinbefore, can be administered for the treatment of various disorders in the form of pharmaceutical compositions.

If the PRO polypeptide is intracellular and whole antibodies are used as inhibitors, internalizing antibodies are preferred. However, lipofections or liposomes can also be used to deliver the antibody, or an antibody fragment, into cells. Where antibody fragments are used, the smallest inhibitory fragment that specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable-region sequences of an antibody, peptide molecules can be designed that retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. See, *e.g.*, Marasco *et al.*, *Proc. Natl. Acad. Sci. USA*, 90: 7889-7893 (1993). The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences, supra*.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT[™] (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be

intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulphhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

5 **L. Tissue Distribution**

The location of tissues expressing the PRO can be identified by determining mRNA expression in various human tissues. The location of such genes provides information about which tissues are most likely to be affected by the stimulating and inhibiting activities of the PRO polypeptides. The location of a gene in a specific tissue also provides sample tissue for the activity blocking assays discussed below.

10 As noted before, gene expression in various tissues may be measured by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, *Proc. Natl. Acad. Sci. USA*, 77:5201-5205 [1980]), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or

15 DNA-protein duplexes.

Gene expression in various tissues, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal.

20 Conveniently, the antibodies may be prepared against a native sequence of a PRO polypeptide or against a synthetic peptide based on the DNA sequences encoding the PRO polypeptide or against an exogenous sequence fused to a DNA encoding a PRO polypeptide and encoding a specific antibody epitope. General techniques for generating antibodies, and special protocols for Northern blotting and *in situ* hybridization are provided below.

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M. Antibody Binding Studies

The activity of the PRO polypeptides can be further verified by antibody binding studies, in which the ability of an anti-PRO antibody to inhibit the effect of the respective PRO polypeptide on tissue cells is tested. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate

30 antibodies, the preparation of which will be described hereinbelow.

Antibody binding studies may be carried out in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, *Monoclonal Antibodies: A Manual of Techniques*, pp.147-158 (CRC Press, Inc., 1987).

Competitive binding assays rely on the ability of a labeled standard to compete with the test

35 sample analyte for binding with a limited amount of antibody. The amount of target protein in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies preferably are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

40 Sandwich assays involve the use of two antibodies, each capable of binding to a different

immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. See, *e.g.*, US Pat No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

For immunohistochemistry, the tissue sample may be fresh or frozen or may be embedded in paraffin and fixed with a preservative such as formalin, for example.

N. Screening Assays for Drug Candidates

Screening assays for drug candidates are designed to identify compounds that bind to or complex with the polypeptides encoded by the genes identified herein or a biologically active fragment thereof, or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules contemplated include synthetic organic or inorganic compounds, including peptides, preferably soluble peptides, (poly)peptide-immunoglobulin fusions, and, in particular, antibodies including, without limitation, poly- and monoclonal antibodies and antibody fragments, single-chain antibodies, anti-idiotypic antibodies, and chimeric or humanized versions of such antibodies or fragments, as well as human antibodies and antibody fragments. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays and cell based assays, which are well characterized in the art.

All assays are common in that they call for contacting the drug candidate with a polypeptide encoded by a nucleic acid identified herein under conditions and for a time sufficient to allow these two components to interact.

In binding assays, the interaction is binding and the complex formed can be isolated or detected in the reaction mixture. In a particular embodiment, the polypeptide encoded by the gene identified herein or the drug candidate is immobilized on a solid phase, *e.g.*, on a microtiter plate, by covalent or non-covalent attachments. Non-covalent attachment generally is accomplished by coating the solid surface with a solution of the polypeptide and drying. Alternatively, an immobilized antibody, *e.g.*, a monoclonal antibody, specific for the polypeptide to be immobilized can be used to anchor it to a solid surface. The assay is performed by adding the non-immobilized component, which may be labeled by a detectable label, to the immobilized component, *e.g.*, the coated surface containing the anchored component. When the reaction is complete, the non-reacted components are removed, *e.g.*, by washing, and complexes anchored on the solid surface are detected. When the originally non-immobilized component carries a detectable label, the detection of label immobilized on the surface indicates that complexing occurred. Where the originally non-immobilized component does not carry a label, complexing can be detected, for example, by using a labelled antibody specifically binding the immobilized complex.

If the candidate compound interacts with but does not bind to a particular protein encoded by a gene identified herein, its interaction with that protein can be assayed by methods well known for detecting protein-protein interactions. Such assays include traditional approaches, such as, cross-linking, co-

immunoprecipitation, and co-purification through gradients or chromatographic columns. In addition, protein-protein interactions can be monitored by using a yeast-based genetic system described by Fields and co-workers [Fields and Song, *Nature (London)* **340**, 245-246 (1989); Chien *et al.*, *Proc. Natl. Acad. Sci. USA* **88**, 9578-9582 (1991)] as disclosed by Chevray and Nathans, *Proc. Natl. Acad. Sci. USA* **89**, 5789-5793 (1991). Many transcriptional activators, such as yeast GAL4, consist of two physically discrete modular domains, one acting as the DNA-binding domain, while the other one functioning as the transcription activation domain. The yeast expression system described in the foregoing publications (generally referred to as the "two-hybrid system") takes advantage of this property, and employs two hybrid proteins, one in which the target protein is fused to the DNA-binding domain of GAL4, and another, in which candidate activating proteins are fused to the activation domain. The expression of a GAL1-*lacZ* reporter gene under control of a GAL4-activated promoter depends on reconstitution of GAL4 activity via protein-protein interaction. Colonies containing interacting polypeptides are detected with a chromogenic substrate for β -galactosidase. A complete kit (MATCHMAKER™) for identifying protein-protein interactions between two specific proteins using the two-hybrid technique is commercially available from Clontech. This system can also be extended to map protein domains involved in specific protein interactions as well as to pinpoint amino acid residues that are crucial for these interactions.

In order to find compounds that interfere with the interaction of a gene identified herein and other intra- or extracellular components can be tested, a reaction mixture is usually prepared containing the product of the gene and the intra- or extracellular component under conditions and for a time allowing for the interaction and binding of the two products. To test the ability of a test compound to inhibit binding, the reaction is run in the absence and in the presence of the test compound. In addition, a placebo may be added to a third reaction mixture, to serve as positive control. The binding (complex formation) between the test compound and the intra- or extracellular component present in the mixture is monitored as described above. The formation of a complex in the control reaction(s) but not in the reaction mixture containing the test compound indicates that the test compound interferes with the interaction of the test compound and its reaction partner.

O. Articles of Manufacture

In another embodiment of the invention, an article of manufacture containing materials (e.g., comprising a PRO molecule) useful for the diagnosis or treatment of the disorders described above is provided. The article of manufacture comprises a container and an instruction. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for diagnosing or treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is usually a polypeptide or an antibody of the invention. An instruction or label on, or associated with, the container indicates that the composition is used for diagnosing or treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including

other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

VI. Assay characterizations: Correlation between *in vitro* assays and *in vivo* therapeutic effect

Recent studies using agarose gel electrophoresis and terminal dUTP nick-end labeling (TUNEL) indicate that photoreceptor cell death occurs principally by apoptosis. Chang, G-Q, Hao Y., Wong F., *Neuron* **11**: 595-605 (1993); Portera-Cailliau, C. *et al.*, *Proc. Nat'l Acad. Sci. USA* **91**: 974-97 (1993); Adler R., *Curr. Top. Dev. Biol.* **16**: 207-252 (1980). These studies examined mouse models of human retinal degeneration (*retinitis pigmentosa*): *rd* mice (which have a mutation in the *b* subunit of cGMP phosphodiesterase); *rds* mice (which have a mutation in peripherin); and transgenic mice, which have a mutation in rhodopsin. In all three models there is a substantial increase in apoptosis at the time of photoreceptor cell death. Apoptosis is also known to be prominent in the RCS rat, as well as in the light-damaged rat retina. Tso M, *et al.*, *Invest. Ophthalmol. Vis. Sci.* **35**: 2693-2699 (1994); Shahinfar S., *et al.*, *Curr. Eye Res.* **10**: 47-59 (1991).

Apoptosis appears to be a tightly controlled "shutdown" process or self-selecting cell suicide which by preventing the leakage of destructive enzymes, allows healthy neighboring cells to continue their normal functioning. Wong, F., *Arch. Ophthalmol.* **113**: 1245-47 (1995). During this process, the cell's outer membrane remains intact as the cell undergoes nuclear condensation, cytoplasmic shrinkage, membrane blebbing, formation of apoptotic bodies, and sometimes DNA fragmentation.

Apoptosis is now believed to play a key role in degenerative diseases of the eye, such as *retinitis pigmentosa* (*rp*). *Rp* is believed to be caused by mutations in the rhodopsin gene, Dryja, TP, *Nature* **343**: 364-366 (1990). In addition, other photoreceptor-specific genetic mutations have been uncovered which induce *rp*, among them the mutants known as retinal degeneration (*rd*), McLaughlin ME, *et al.* *Nat. Genet.* **4**: 30-134 (1993), and retinal degeneration slow (*rds*), Farrar G.J. *et al.*, *Nature*, **354**: 478-80 (1991); Kajiwar K. *et al.*, *Nature* **354**: 480-83 (1991). It has further been discovered that the autosomal dominant types of *rp* may be caused by any one of more than 70 mutations of the rhodopsin gene. Humphries, P. *et al.*, *Science* **256**: 804-808 (1992); Dryja, T.P. *et al.*, *Invest. Ophthalmol. Vis. Sci.* (1995), **36**: 1197-1200 (1995). Rhodopsin mutations are known to be the basis of autosomal recessive *rp* in some families as well. Rosenfeld, P.J. *et al.*, *Nat. Genet.* **1**: 209-13 (1992); Kumaramanickavel, G. *et al.*, **8**: 10-11 (1994). As a result, the rhodopsin gene is now considered an archetypal model for the study of *rp*.

The role of apoptosis in *rp* has been observed in mouse photoreceptors. Several lines of transgenic mice which express mutant rhodopsin have been created, and as a result, can simulate a form of the autosomal dominant *rp* found in humans. These animal models exhibit dying photoreceptors through various characteristics of apoptosis, including morphological changes and DNA fragmentation. Chang C-G *et al.*, *Neuron* (1993), **11**: 595-605; Portera-Cailliau C. *et al.*, *Proc. Natl. Acad. Sci. USA* (1994), **91**: 974-978. Along with other experimental results, these findings have led researches to the conclusion that apoptosis is a major mechanism of murine photoreceptor death, as it is induced not only by mutations in the rhodopsin gene, but also by mutations in the *rd* and *rds* genes. Chang C-G *et al.*, *supra*, Portera-Cailliau C. *et al.*, *supra*, Lolley R.N. *et al.*, *Invest. Ophthalmol. Vis. Sci.* (1994), **35**: 358-362.

Of great interest is the observation that photoreceptor degeneration occurs through apoptosis in response not only to genetic abnormalities, but also after experimental retina detachment. Cook, BE *et al.*, *Invest. Ophthalmol. Vis. Sci.* (1995), 36: 990-996. Moreover, apoptotic cell death was also observed in acute retinal lesions in the albino rat induced by relatively low light levels and short exposure duration (1000 & 3000 lux, diffuse, white light for 2 hours), Remé *et al.*, *Degenerative Diseases of the Retina*, Anderson R.E. *et al.*, eds, Plenum Press, pp. 19-25 (1995). This discovery has lead to the search for survival-promoting trophic factors, factors which are believed to become unavailable to photoreceptors when the subretinal space expands and the composition of the interphotoreceptor matrix changes as a consequence of retinal detachment. Chader G.J. (1989), *Invest. Ophthalmol. Vis. Sci.* 30: 7-22; Berman E.R., *Biochemistry of the Eye* (1991), New York, NY, Plenum Press; Steinberg R.H., *Curr. Opin. Neurobiol.* 4: 515-24.

The death of photoreceptor cells through apoptosis is indicative that rather than being passive victims of the cumulative effects of mutations, photoreceptors die in genetic disorders such as *retinitis pigmentosa* by activation of their own "cell-death program." Adler, R. (1996) *Arch. Ophthalmol.* 114: 79-83. This implies that there is a role which certain neurotrophic factors and related molecules play in the degeneration of cones resulting from mutations in rod proteins.

The following examples are demonstrative of therapeutic utility because cell death occurs via apoptosis, the same mechanism as has been shown to occur in various retinal degenerative disorders. The knowledge that known growth factors prevented apoptosis correlated with preserved vision in animal models is indicative that prospective factors which prevent apoptosis would also have therapeutic utility in retinal degenerative disorders.

The following examples are offered by way of illustration and not by way of limitation. The disclosures of all citations in the specification are expressly incorporated herein by reference.

EXAMPLES

Example 1

A. Isolation of cDNA Clones Encoding Human PRO175 (UNQ149)

The native sequence PRO175 polypeptide of Figure 2 (SEQ ID NO:2) is described and characterized in Gurney *et al.*, *Curr. Biol.* 9(4): 215-8 (1999). A DNA sequence encoding SEQ ID NO:2 is further available as GenBank Accession number AF125303.

The nucleotide sequence of DNA19355-1150 is shown in Figure 1 (SEQ ID NO:1). Clone DNA19355-1150 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 21-23 (Fig. 1; SEQ ID NO:1). The predicted polypeptide precursor is 177 amino acids long and has a calculated molecular weight of approximately 20,308 daltons (Figure 2). Hydropathy analysis suggests the presence of a signal sequence about amino acid residues 1 to 44. Two potential N-linked glycosylation sites have been identified at position 129 (Asn) and position 161 (Asn) of the sequence shown in Fig. 2 (SEQ ID NO:2). Clone DNA19355-1150 has been deposited with ATCC on November 18, 1997 and is assigned ATCC deposit no. 209466. The native sequence PRO175 polypeptide of SEQ ID

NO:2 is obtained or obtainable by expressing the molecule encoded by the cDNA insert of the sequence ATCC 209466.

B. Isolation of cDNA Clones Encoding Human PRO220 (UNQ194)

5 The extracellular domain (ECD) sequences (including the secretion signal, if any) of from about 950 known secreted proteins from the Swiss-Prot public protein database were used to search expressed sequence tag (EST) databases. The EST databases included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 (Altschul *et al.*, *Methods in Enzymology*
10 266:460-480 (1996)) as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequence. Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington).

15 A consensus DNA sequence was assembled relative to the other identified EST sequences, which was designated herein as DNA28749 (SEQ ID NO:3). Based on the DNA28749 (SEQ ID NO:3), oligonucleotides were synthesized to identify by PCR a cDNA library that contained the sequence of interest and for use as probes to isolate a clone of the corresponding full-length PRO220 encoding sequence.

The oligonucleotide primers and probes used in the above procedure were:

forward PCR primer: 5'-TCACCTGGAGCCTTTATTGGCC-3' (SEQ ID NO:4)
20 reverse PCR primer: 5'-ATACCAGCTATAACCAGGCTGCG-3' (SEQ ID NO:5)
hybridization probe: (SEQ ID NO:6)
5'-CAACAGTAAGTGTTTGATGCTCTCCAAATCTAGAGATTCTGATGATTGGG-3'

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used
25 to isolate clones encoding the PRO220 gene using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal lung tissue. The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized
30 appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; Holmes *et al.*, *Science*, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence DNA32298-1132 (SEQ ID NO:7), which encodes the native sequence PRO220 protein of Figures 4 (SEQ
35 ID NO:8).

The entire nucleotide sequence of DNA32298-1132 is shown in Figure 3 (SEQ ID NO:7). Clone DNA32298-1132 (SEQ ID NO:7) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 480-482 and ending at the stop codon at nucleotide positions 2604-2606. The predicted polypeptide precursor is 708 amino acids long (Figure 4). Hydropathy analysis
40 suggests the presence of a signal peptide as residues 1-22, a transmembrane domain at about amino acid residues 632 to 651, and a tyrosine kinase phosphorylation site at about amino acid residue 570 and a cell

attachment sequence site is present at residue 277. Clone DNA32298-1133 has been deposited with the ATCC on September 16, 1997 and is assigned ATCC deposit no. 209257.

C. Isolation of cDNA Clones Encoding Human PRO216 (UNQ190)

A procedure analogous to the one above for the isolation of native sequence PRO220 (SEQ ID NO:8) can be employed to isolate DNA33087-1158 (SEQ ID NO:9) (Figure 5) which encodes the native sequence PRO216 protein of Figure 6 (SEQ ID NO:10). Hydropathy analysis suggests the presence of a signal sequence at amino acid residues 1 to 20, tyrosine kinase phosphorylation sites at amino acid residues 268-274 and 300-306, and N-myristoylation site residue 230-235, and leucine zippers at residues 146 to 167 and 217 to 238. An alternative to a traditional isolation technique, the DNA sequence is publicly available from GenBank as accession number AB000114 which encodes Dayhoff protein AB000114_1. Alternatively still, the sequence is described in *Ohno et al., Biochem. Biophys. Res. Commun.* **228**(2): 411-414 (1996). Clone DNA33087-1158 has been deposited with the American Type Culture Collection (ATCC) on September 16, 1997 and has been assigned ATCC Dep. No. 209381.

D. Isolation of cDNA Clones Encoding Human PRO243 (UNQ217)

Introduction:

Three P1 clones and one PAC clones (Genome Systems Inc., St. Louis, MO; cat. Nos. P1-2535 and PAC-6539) encompassing the human thrombopoietin (THPO) locus were isolated and a 140 kb region was sequenced using the ordered shotgun strategy (Chen *et al., Genomics* **17**: 651-656 (1993)), coupled with a PCR-based gap filling approach. Analysis revealed that the region is gene-rich with four additional genes located very close to THPO: tumor necrosis factor-receptor type 1 associated protein 2 (TRAP2) and elongation initiation factor gamma (eIF4g), chloride channel 2 (CLCN2) and RNA polymerase II subunit hRPB17. While no THPO homolog was found in the region, four novel genes have been predicted by computer-assisted gene detection (GRAIL)(Xu *et al., Gen. Engin.* **16**: 241-253 (1994), the presence of CpG islands (Cross, S. and Bird, A., *Curr. Opin. Genet. & Devel.* **5**: 109-314 (1995), and homology to known genes (as detected by WU-BLAST2.0)(Altschul and Gish, *Methods Enzymol.* **266**: 460-480 (1996).

Procedures:

P1 and PAC clones:

The initial human P1 clone was isolated from a genomic P1 library (Genome Systems Inc., St. Louis, MO; cat. no.: P1-2535) screened with PCR primers designed from the THPO genomic sequence (A.L. Gurney, *et al., Blood* **85**: 981-88 (1995). PCR primers were designed from the end sequences derived from this P1 clone were then used to screen P1 and PAC libraries (Genome Systems, Cat. Nos.: P1-2535 & PAC-6539) to identify overlapping clones.

Ordered Shotgun Strategy:

The Ordered Shotgun Strategy (OSS) (Chen *et al., Genomics* **17**: 651-656 (1993)) involves the mapping and sequencing of large genomic DNA clones with a hierarchical approach. The P1 or PAC clone was sonicated and the fragments subcloned into lambda vector (λ Bluestar) (Novagen, Inc., Madison, WI; cat. no. 69242-3). The lambda subclone inserts were isolated by long-range PCR (Barnes, W. *Proc. Natl. Acad. Sci. USA* **91**: 2216-2220 (1994) and the ends sequenced. The lambda-end sequences were overlapped

to create a partial map of the original clone. Those lambda clones with overlapping end-sequences were identified, the insets subcloned into a plasmid vector (pUC9 or pUC18) and the ends of the plasmid subclones were sequenced and assembled to generate a contiguous sequence. This directed sequencing strategy minimizes the redundancy required while allowing one to scan for and concentrate on interesting regions.

In order to define better the THPO locus and to search for other genes related to the hematopoietin family, four genomic clones were isolated from this region by PCR screening of human P1 and PAC libraries (Genome System, Inc., Cat. Nos.: P1-2535 and PAC-6539). The sizes of the genomic fragments are as follows: P1.t is 40 kb; P1.g is 70 kb; P1.u is 70 kb; and PAC.z is 200 kb. The relationships between these four genomic clones are illustrated in Figure 7. Approximately 80% of the 200 kb genomic DNA region was sequenced by the Ordered Shotgun Strategy (OSS) (Chen *et al.*, *Genomics* 17: 651-56 (1993) (Figure 9), and assembled into contigs using AutoAssembler™ (Applied Biosystems, Perkin Elmer, Foster City, CA, cat. no. 903227). The preliminary order of these contigs was determined by manual analysis. There were 46 contigs and gap filling was employed. Table 7 summarized the number and sizes of the gaps.

Table 7:

Summary of the gaps in the 140 kb region

Size of gap	number
<50 bp	13
50-150 bp	7
150-300 bp	7
300-1000 bp	10
1000-5000 bp	7
> 5000 bp	2 (=15,000 bp)

DNA sequencing:

ABI DYE-primer™ chemistry (PE Applied Biosystems, Foster City, CA; Cat. No.: 402112) was used to end-sequence the lambda and plasmid subclones. ABI DYE-terminator™ chemistry (PE Applied Biosystems, Foster City, CA, Cat. No: 403044) was used to sequence the PCR products with their respective PCR primers. The sequences were collected with an ABI377 instrument. For PCR products larger than 1kb, walking primers were used. The sequences of contigs generated by the OSS strategy in AutoAssembler™ (PE Applied Biosystems, Foster City, CA; Cat. No: 903227) and the gap-filling sequencing trace files were imported into Sequencer™ (Gene Codes Corp., Ann Arbor, MI) for overlapping and editing.

PCR-Based gap filling Strategy:

Primers were designed based on the 5'- and 3'-end sequenced of each contig, avoiding repetitive and low quality sequence regions. All primers were designed to be 19-24-mers with 50-70% G/C content. Oligos were synthesized and gel-purified by standard methods.

Since the orientation and order of the contigs were unknown, permutations of the primers were used in the amplification reactions. Two PCR kits were used: first, XL PCR kit (Perkin Elmer, Norwalk, CT; Cat. No.: N8080205), with extension times of approximately 10 minutes; and second, the Taq polymerase PCR kit (Qiagen Inc., Valencia, CA; Cat. No.: 201223) was used under high stringency conditions if smeared or multiple products were observed with the XL PCR kit. The main PCR product

from each successful reactions was extracted from a 0.9% low melting agarose gel and purified with the GeneClean DNA Purification kit prior to sequencing.

Analysis:

5 The identification and characterization of coding regions was carried out as follows: First, repetitive sequences were masked using RepeatMasker (A.F.A. Smit & P. Green, <http://ftp.genome.washington.edu>) which screens DNA sequences in FastA format against a library of repetitive elements and returns a masked query sequence. Repeats not masked were identified by comparing the sequence to the GenBank database using WUBLAST (Altschul, S & Gish, W., *Methods Enzymol.* **266**: 460-480 (1996) and were masked manually.

10 Next, known genes were revealed by comparing the genomic regions against Genentech's protein database using the WUBLAST2.0 algorithm and then annotated by aligning the genomic and cDNA sequences for each gene, respectively, using a Needleman-Wunch (Needleman and Wunsch, *J. Mol. Biol.* **48**: 443-453 (1970) algorithm to find regions of local identity between sequences which are otherwise largely dissimilar. The strategy results in detection of all exons of the five known genes in the region, THPO, TRAP2, eIF4g, CLCN2 and hRPB17 (Table 8).

Table 8:

Summary of known genes located in the 140 kb region analyzed

Known genes	Map position
eukaryotic translation initiation factor 4 gamma	3q27-qter
thrombopoietin	3q26-q27
chloride channel 2	3q26-qter
TNF receptor associated protein 2	not previously mapped
RNA polymerase II subunit hRPB17	not previously mapped
<p>25 Finally, novel transcription units were predicted using a number of approaches. CpG islands (S. Cross & Bird, A., <i>Curr. Opin. Genet. Dev.</i> 5: 109-314 (1995) islands were used to define promoter regions and were identified as clusters of sites cleaved by enzymes recognizing GC-rich, 6 or 8-mer palindromic sequences. CpG islands are usually associated with promoter regions of genes. WUBLAST2.0 analysis of short genomic regions (10-20 kb) versus GenBank revealed matches to ESTs. The individual EST sequences (or where possible, their sequence chromatogram files) were retrieved and assembled with Sequencher to provide a theoretical cDNA sequence (DNA34415). GRAIL2 (ApoCom Inc., Knoxville, TN, command line version for the DEC alpha) was used to predict a novel exon. The five known genes in the region served as internal controls for the success of the GRAIL algorithm.</p>	

Isolation:

35 Chordin cDNA clones were isolated from an oligo-dT-primed human fetal lung library. Human fetal lung polyA⁺ RNA was purchased from Clontech (cat #6528-1, lot #43777) and 5 mg used to construct a cDNA library in pKR5B (Genentech, LIB26). The 3'-primer (pGACTAGTTCTAGATCGCGAGCGGCCCTTTT) (SEQ ID NO:11) and the 5'-linker (pCGGACGCGTGGGGCCTGCGCACCCAGCT) (SEQ ID NO:12) were designed to introduce SalI and NotI restriction sites. Clones were screened with oligonucleotide probes designed from the putative

human chordin cDNA sequence (DNA34415) (SEQ ID NO:13) deduced by manually "splicing" together the proposed genomic exons of the gene. PCR primers flanking the probes were used to confirm the identity of the cDNA clones prior to sequencing, resulting in the isolation of DNA35917-1207 (SEQ ID NO:14) and the encoded native sequence PRO243 polypeptide of Figure 9 (SEQ ID NO:15).

5 The screening oligonucleotides probes used were the following:

OLI5640 34415.p1 5'-GCCGCTCCCCGAACGGGCAGCGGCTCCTTCTCAGAA-3' (SEQ ID NO:16)

OLI5642 34415.p2 5'-GGCGCACAGCACGCAGCGCATCACCCGAATGGCTC-3' (SEQ ID NO:17);

and the flanking probes used were the following:

OLI5639 34415.fl 5'-GTGCTGCCCATCCGTTCTGAGAAGGA-3' (SEQ ID NO:18)

10 OLI5643 34415.r 5'-GCAGGGTGCTCAAACAGGACAC-3' (SEQ ID NO:19).

The native sequence PRO243 polypeptide of Figure 9 (SEQ ID NO:15) contains a putative signal peptide at amino acid residues 1-23, tyrosine kinase phosphorylation sites at amino acid residues 145 to 152 and 778 to 785, a cell attachment sequence at residues 87-90, a cell attachment sequence at residues 165-167 and a leucine zipper at residues 315-336.

15 Clone DNA35917-1207 has been deposited with the ATCC on December 3, 1997 and has been assigned ATCC Dep. No. 209508.

E. Isolation of cDNA Clones Encoding Human PRO306 (UNQ269)

The extracellular domain (ECD) sequences (including the secretion signal, if any) of from about
20 950 known secreted proteins from the Swiss-Prot public protein database were used to search expressed sequence tag (EST) databases. The EST databases included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 (Altschul *et al.*, *Methods in Enzymology* 266: 460-480 (1996)) as a comparison of the ECD protein sequences to a 6 frame translation of the EST
25 sequences. Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington).

A consensus DNA sequence was assembled relative to other EST sequences using phrap. This consensus sequence was designated DNA36458 (SEQ ID NO:20). This consensus DNA sequence was
30 extended using repeated cycles of BLAST and phrap to extend DNA36458 (SEQ ID NO:20) as far as possible using the sources of EST sequences discussed above. The extended assembly and DNA36458 (SEQ ID NO:20) were used as templates to synthesize oligonucleotides to: 1) identify by PCR cDNA library that contained the sequence of interest, and 2) use as probes to isolate a clone of the full-length coding sequence for PRO306. Forward and reverse PCR primers generally range from 20 to 30 nucleotides and are
35 often designed to give a PCR product of about 100-1000 bp in length. The probe sequences are typically 40-55 bp in length. In some cases, additional oligonucleotides are synthesized when the consensus sequence is greater than about 1-1.5 kbp. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel *et al.*, *Current Protocols in Molecular Biology*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest by
40 the *in vivo* cloning procedures using the probe oligonucleotide and one of the primer pairs.

The forward, reverse and hybridization probes in the above procedure were the following:

- forward PCR primer (.f1): 5'-CAGGTCGAACCCAGACCACGATGC-3' (SEQ ID NO:21)
 forward PCR primer (.f2): 5'-GCCACATGGCCCAGCTTG-3' (SEQ ID NO:22)
 forward PCR primer (.f3): 5'-GAGACGGAGGAAGCAGGC-3' (SEQ ID NO:23)
 5 forward PCR primer (.f11): 5'-GGCCACACTTACAGCTCTG-3' (SEQ ID NO:24)
 reverse PCR primer (.r1): 5'-AGCCGGCTTCTGAGGGCGTCTACC-3' (SEQ ID NO:25)
 hybridization probe: (SEQ ID NO:26)
 5'-TGGTGCTGCCGCTGCTGCTCCTGGCCGCGGCAGCCCTGGCCGAAG-3'

10 In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with one of the PCR primer pairs identified above. A positive library was then used to isolate clones encoding the PRO306 gene using the probe oligonucleotide and one of the PCR primers.

15 RNA for construction of the cDNA libraries was isolated from human fetal kidney tissue. The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes *et al.*, *Science* 253: 1278-1280 (1991)) in the unique XhoI and NotI sites.

20 DNA sequencing of the clones isolated as described above gave the full-length sequence DNA39984-1221 (SEQ ID NO:27) and the derived native sequence PRO306 polypeptide of Figure 11 (SEQ ID NO:28). DNA39984-1221 (SEQ ID NO:27) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 199-201 and ending at the stop codon at nucleotide positions 1471-1473 (Figure 10). The predicted native sequence PRO306 polypeptide precursor is 424
 25 amino acids long (Figure 22). The native sequence PRO306 polypeptide of Figure 11 (SEQ ID NO:28) contains a putative signal peptide at amino acid residues 1-22, glycosaminoglycan attachment sites at residues 388 to 390 and tyrosine kinase phosphorylation sites at residues 62-69. Clone DNA39984-1221 has been deposited with the ATCC on November 7, 1997 as ATCC Dep. No. 209435.

30 Alternatively, DNA encoding a polypeptide matching 423 amino acid residues of SEQ ID NO:28) is available from GenBank as accession number D87465 which encodes the Dayhoff polypeptide D87465_1. This sequence is further described and characterized in Vannahme *et al.*, *J. Neurochem.* 73(1): 12-20 (1999). This same sequence has also been submitted to GenBank as accession number HSAJ1453 which encodes the Dayhoff polypeptide HSAJ1453_1. This sequence is also discussed in the publication Nagase *et al.*, *DNA Res.* 3 (5): 321-329 (1996).

35

F. Isolation of cDNA Clones Encoding Human PRO346 (UNQ305)

The extracellular domain (ECD) sequences (including the secretion signal sequence, if any) from about 950 known secreted proteins from the Swiss-Prot public database were used to search Expressed sequence tag (EST) databases. The EST databases included public EST databases (e.g., GenBank, Dayhoff),
 40 and proprietary ESTs from Genentech isolated using the procedure described previously. The search was

performed using the computer program BLAST or BLAST2 [Altschul *et al.*, *Methods in Enzymology*, 266:460-480 (1996)] as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequences. Those comparisons resulting in a BLAST score of 70 (or in some cases, 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington).

The EST sequence (H68654, DNA 38240)(SEQ ID NO:29) was identified. A consensus DNA sequence was isolated using repeated cycles of blast and phrap to extend the consensus sequence as far as possible using the public databases GenBank, Dayhoff as well as a proprietary Genentech database comprised of sequencing isolated the procedure described previously.

The consensus and extended consensus assembly served as a basis for the creation of oligonucleotides. These oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence. Forward and reverse PCR primers (notated as *.f and *.r, respectively) may range from 20 to 30 nucleotides (typically about 24), and are designed to give a PCR product of 100-1000 bp in length. The probe sequences (notated as *.p) are typically 40-55 bp (typically about 50) in length. In some cases, additional oligonucleotides are synthesized when the consensus sequence is greater than 1-1.5 kbp. In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel *et al.*, *Current Protocols in Molecular Biology*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest by the *in vivo* cloning procedure using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal liver. The cDNA libraries used to isolated the cDNA clones were constructed by standard methods using commercially available reagents (*e.g.*, Invitrogen, San Diego, CA; Clontech, *etc.*) The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes *et al.*, *Science*, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

A cDNA clone was isolated and sequenced in entirety. The entire nucleotide sequence of DNA44167-1243 is shown in Figure 12 (SEQ ID NO:30). Clone DNA44167-1243 (SEQ ID NO:30) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 64-66 (Fig. 12; SEQ ID NO:30). The predicted polypeptide precursor is 450 amino acids long (Figure 13)(SEQ ID NO:31). SEQ ID NO:31 has a putative signal peptide at amino acid residues 1-18, a transmembrane domain at about residues 340 to 358, a tyrosine kinase phosphorylation site at residues 272 to 279 and a prokaryotic membrane lipoprotein lipid attachment site at residues 7 to 17.

Clone DNA44167-1243 has been deposited with ATCC on November 7, 1997 and is assigned ATCC deposit no. 209434.

The oligonucleotide sequences used in the above procedure were the following:

OLI2691 (38240.f1) (SEQ ID NO:32)
5'-GATCCTGTCACAAAGCCAGTGGTGC-3'

OLI2693 (38240.r1) (SEQ ID NO:33)
5'-CACTGACAGGGTTCCTCACCCAGG-3'

OL12692 (38240.p1)

(SEQ ID NO:34)

5'-CTCCCTCTGGGCTGTGGAGTATGTGGGGAACATGACCCTGACATG-3'

G. Isolation of cDNA Clones Encoding Hman PRO322 (UNQ283)

5 The extracellular domain (ECD) sequences (including the secretion signal, if any) of from about 950 known secreted proteins from the Swiss-Prot public protein database were used to search expressed sequence tag (EST) databases. The EST databases included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 (Altschul *et al.*, *Methods in Enzymology* 10 266:460-480 (1996)) as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequence. Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington).

15 A consensus DNA sequence was assembled relative to two EST sequences using phrap. This consensus sequence is herein designated DNA36951 (SEQ ID NO:35). Also used were the two EST sequences, "2604309" (SEQ ID NO:36) and "1968970" (SEQ ID NO:37), both from Incyte Pharmaceuticals. In addition, the consensus DNA sequence was extended using repeated cycles of BLAST and phrap to extend the consensus sequence as far as possible using the sources of EST sequences discussed above.

20 Based on the consensus sequence DNA36951 (SEQ ID NO:35), oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for the PRO322. Forward and reverse PCR primers generally range from 20 to 30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probe sequences are typically 40-55 bp in length. In some cases, additional oligonucleotides are synthesized when the consensus sequence is greater than about 1-1.5kbp. In order to 25 screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel *et al.*, *Current Protocols in Molecular Biology*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

A pair of PCR primers (forward and reverse) were synthesized:

30 forward PCR primer 5'-CAGCCTACAGAATAAAGATGGCCC-3' (SEQ ID NO:38)

reverse PCR primer 5'-GGTGCAATGATCTGCCAGGCTGAT-3' (SEQ ID NO:39)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus sequence which had the following nucleotide sequence:

35 hybridization probe (SEQ ID NO:40)
5'-AGAAATACCTGTGGTTCAGTCCATCCCAAACCCCTGCTACAACAGCAG-3'

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO322 gene using the probe oligonucleotide and one of the PCR primers.

40 RNA for construction of the cDNA libraries was isolated from human fetal kidney tissue (LIB227). The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed

with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes *et al.*, *Science*, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence DNA48336-1309 (SEQ ID NO:41) and the encoded native sequence PRO322 protein sequence of Figure 15 (SEQ ID NO:42). The entire nucleotide sequence of DNA48336-1309 is shown in Figure 14 (SEQ ID NO:41). Clone DNA48336-1309 (SEQ ID NO:41) contains a single open reading frame with an apparent translational initiation site at about nucleotide positions 166-168 and ending at the stop codon at about nucleotide positions 946-948 (Figure 14). The predicted polypeptide precursor is 260 amino acids long (Figure 15). The full-length, native sequence PRO322 protein shown in Figure 15 (SEQ ID NO:42) has an estimated molecular weight of about 28,028 daltons and a pI of about 7.87. Clone DNA48336-1309 has been deposited with ATCC on March 11, 1998 and is assigned ATCC deposit no. 209669.

SEQ ID NO:42 contains a potential N-glycosylation site is at amino acid residue 110. A serine protease, trypsin family and histidine active site is believed to be present at about amino acid residues about 69 through about 74. A kringle domain proteins motif is at about amino acids about 205 through about 217. A region having homology to serine protease is present about residues 207 to 217 while a tyrosine kinase phosphorylation site is believed present at residues 182 to 188. The putative signal peptide is encoded at about amino acid residues 1-23.

H. Isolation of cDNA Clones Encoding Human PRO536 (UNQ337)

DNA49142-1430 (SEQ ID NO:43) was identified by applying a proprietary signal sequence finding algorithm developed by Genentech, Inc. (South San Francisco, CA) upon ESTs as well as clustered and assembled EST fragments from public (e.g., GenBank) and/or private (LIFESEQ®, Incyte Pharmaceuticals, Inc., Palo Alto, CA) databases. The signal sequence algorithm computes a secretion signal score based on the character of the DNA nucleotides surrounding the first and optionally the second methionine codon(s) (ATG) at the 5'-end of the sequence or sequence fragment under consideration. The nucleotides following the first ATG must code for at least 35 unambiguous amino acids without any stop codons. If the first ATG has the required amino acids, the second is not examined. If neither meets the requirement, the candidate sequence is not scored. In order to determine whether the EST sequence contains an authentic signal sequence, the DNA and corresponding amino acid sequences surrounding the ATG codon are scored using a set of seven sensors (evaluation parameters) known to be associated with secretion signals.

Use of the above described signal sequence algorithm allowed identification of an EST cluster sequence from the Incyte database, designated DNA48351 (SEQ ID NO:44). This EST cluster sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) and a proprietary EST DNA database (Lifeseq®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. The homology search was performed using the computer program BLAST or BLAST2 (Altschul *et al.*, *Methods in Enzymology* 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known

proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). In light of the sequence homology between the DNA48351 sequence (SEQ ID NO:44) and the Merck EST clone no. H11129 (SEQ ID NO:45), the Merck EST clone H11129 was purchased and the cDNA insert was obtained and sequenced. The sequence of this cDNA insert is shown in Figure 16 and is herein designated as DNA49142-1430 (SEQ ID NO:43).

DNA49142-1430 (SEQ ID NO:43) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 48-50 and ending at the stop codon at nucleotide positions 987-989 (Figure 16). The predicted polypeptide precursor is 313 amino acids long (Figure 17)(SEQ ID NO:46). The full-length, native sequence PRO536 protein shown in Figure 17 has an estimated molecular weight of about 34,189 daltons and a pI of about 4.8. Analysis of the full-length, native sequence PRO536 polypeptide (SEQ ID NO:46) shown in Figure 17 evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 25, a potential N-glycosylation site from about amino acid 45 to about amino acid 48 and an amino acid sequence block having homology to sulfatase proteins from about amino acid 16 to about amino acid 26. Clone DNA49142-1430 has been deposited with ATCC on June 23, 1998 and is assigned ATCC deposit no. 203002.

I. Isolation of cDNA Clones Encoding Human PRO943 (UNQ480)

The extracellular domain (ECD) sequences (including the secretion signal sequence, if any) from about 950 known secreted proteins from the Swiss-Prot public database were used to search EST databases. The EST databases included public EST databases (e.g., GenBank) and a proprietary EST database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 [Altschul *et al.*, *Methods in Enzymology*, 266:460-480 (1996)] as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequences. Those comparisons resulting in a BLAST score of 70 (or in some cases, 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington).

A consensus DNA sequence encoding was assembled relative to other EST sequences related by homology using phrap. This consensus sequence was then extended using repeated cycles of BLAST and phrap to extend the consensus sequence as far as possible using the sources of EST sequences discussed above. The extended consensus sequence was designated DNA36360 (SEQ ID NO:47).

Based on the DNA36360 consensus sequence (SEQ ID NO:47), oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest; and 2) for use as probes to isolate the full-length, native sequence PRO943 clone (SEQ ID NO:48) of Figure 19. Forward and reverse PCR primers generally range from 20 to 30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probe sequences are typically 40-55 bp in length. In some cases, additional oligonucleotides are synthesized when the consensus sequence is greater than about 1-1.5 kbp. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel *et al.*, *Current Protocols in Molecular Biology*, *supra*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe

oligonucleotide and one of the primer pairs. The identity of the amplified DNA was confirmed with a hybridization probe.

The PCR primers (forward and reverse) and hybridization probe synthesized for use in the above procedure were:

- 5 forward PCR primer (36360.f1) 5'-CGAGATGACGCCGAGCCCCC-3' (SEQ ID NO:50)
 reverse PCR primer (36360.r1) 5'-CGGTTTCGACACGCGGCAGGTG-3' (SEQ ID NO:51)
 hybridization probe (36360.p1)
 5'-TGCTGCTCCTGCTGCCGCCGCTGCTGCTGGGGGCCTTCCCGCCGG-3' (SEQ ID NO:52)

- 10 In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO943 gene using the probe oligonucleotide and one of the PCR primers.

- 15 RNA for construction of the cDNA libraries was isolated from human fetal brain tissue. The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes *et al.*, *Science*, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

- 20 DNA sequencing of the clones isolated as described above gave a full-length DNA sequence DNA52192-1369 (SEQ ID NO:49) (Figure 18). Clone DNA52192-1369 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 150-152 and ending at the stop codon at nucleotide positions 1662-1664 (Figure 19)(SEQ ID NO:49). The predicted polypeptide precursor is 504 amino acids long (Figure 19)(SEQ ID NO:48). The full-length, native sequence PRO943 protein shown in Figure 50 (SEQ ID NO:48) has an estimated molecular weight of about 54,537 daltons and a pI of about 10.04. Further analysis of SEQ ID NO:48 evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 17, a transmembrane domain from about amino acid 376 to about amino acid 396 (*i.e.*, extracellular domain from about 18-375; intracellular domain 376-504), tyrosine kinase phosphorylation sites from about amino acid 212 to about amino acid 219 and from about amino acid 329 to about amino acid 336, potential N-glycosylation sites from about amino acid 111 to about amino acid 114, from about amino acid 231 to about amino acid 234, from about amino acid 255 to about amino acid 258 and from about amino acid 293 to about amino acid 296 and an immunoglobulin and MHC protein sequence homology block from about amino acid 219 to about amino acid 236. Clone DNA52192-1369 has been deposited with ATCC on July 1, 1998 and is assigned ATCC deposit no. 203042.

35 J. Isolation of cDNA Clones Encoding Human PRO840 (UNQ433)

- 40 A procedure analogous to the one described above for the isolation of PRO220 can be employed to isolate the DNA53987-1438 (SEQ ID NO:53) (Figure 20) which encodes the PRO840 native sequence protein of Figure 21 (SEQ ID NO:54). Hydropathy analysis of SEQ ID NO:54 suggests a signal peptide at about amino acid residues 1-46, a transmembrane domain at about residues 319-339 and a tyrosine kinase phosphorylation site at residues 43-51. Alternatively, similar DNA sequences are available from GenBank

as accession number AF036364 and AJ000534. The GenBank sequence AF036364 is also described by McNally *et al.*, in *FEBS Lett.* 422 (1), 27-32 (1998). Clone DNA53987-1438 has been deposited with the American Type Culture Collection (ATCC) on May 12, 1998 and has been assigned ATCC Dep. No. 209858.

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K. Isolation of cDNA Clones Encoding Human PRO828 (UNQ469)

The extracellular domain (ECD) sequences (including the secretion signal, if any) of from about 950 known secreted proteins from the Swiss-Prot public protein database were used to search expressed sequence tag (EST) databases. The EST databases included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 (Altshul *et al.*, *Methods in Enzymology* 266:460-480 (1996)) as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequence. Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington).

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A consensus DNA sequence was identified using the above described analysis which appeared to encode a fragment of a potential secreted protein. This sequence was extended using repeated cycles of BLAST and phrap to extend the consensus sequence as far as possible using the sources of EST sequences discussed above. The extended assembly sequence is referred to herein as DNA35717 (SEQ ID NO:55).

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The DNA35717 consensus sequence (SEQ ID NO:55) served as a basis to for oligonucleotide synthesis: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length PRO828 encoding sequence. Forward and reverse PCR primers generally range from 20 to 30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probe sequences are typically 40-55 bp in length. In some cases, additional oligonucleotides are synthesized when the consensus sequence is greater than about 1-1.5kbp. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel *et al.*, *Current Protocols in Molecular Biology*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

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The synthesized forward and reverse PCR primers, and the hybridization probe for use with the above isolation method were:

forward PCR primer (35717.fl): 5'-GCAGGACTTCTACGACTTCAAGGC-3' (SEQ ID NO:56)

reverse PCR primer (35717.r1): 5'-AGTCTGGGCCAGGTACTTGAAGGC-3' (SEQ ID NO:57)

hybridization probe (35717.p1): (SEQ ID NO:58)

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5'-CAACATCCGGGGCAAACCTGGTGTCTGCTGGAGAAGTACCGCGGATCGGTGT-3'

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO828 gene using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal lung tissue (LIB25). The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using

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commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes *et al.*, *Science*, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

DNA sequencing of the clones isolated as described above gave DNA57037-1444 (SEQ ID NO:59) and the encoded native sequence PRO828 protein sequence of Figure 23 (SEQ ID NO:60).

The entire nucleotide sequence of DNA57037-1444 is shown in Figure 22 (SEQ ID NO:59). Clone DNA57037-1444 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 34-36 and ending at the stop codon at nucleotide positions 595-597 (Figure 22)(SEQ ID NO:59). The predicted polypeptide precursor is 187 amino acids long (Figure 23)(SEQ ID NO:60). The full-length, native sequence PRO828 protein shown in Figure 23 (SEQ ID NO:60) has an estimated molecular weight of about 20,996 daltons and a pI of about 8.62. Analysis of SEQ ID NO:60 evidences the presence of the following: a signal peptide at about amino acids 1-21; sequences identity to glutathione peroxidases signature 2 at about amino acids 82-89; sequence identity to glutathione peroxidases selenocysteine proteins at about amino acids 35-60, 63-100, 107-134, and 138-159.

Clone DNA57037-1444 has been deposited with ATCC on May 27, 1998, and has been assigned ATCC deposit no. 209903.

L. Isolation of cDNA Clones Encoding Human PRO826 (UNQ467)

DNA57694-1341 (SEQ ID NO:64) was identified by applying a proprietary signal sequence finding algorithm developed by Genentech, Inc. (South San Francisco, CA) upon ESTs as well as clustered and assembled EST fragments from public (e.g., GenBank) and/or private (LIFESEQ®, Incyte Pharmaceuticals, Inc., Palo Alto, CA) databases. The signal sequence algorithm computes a secretion signal score based on the character of the DNA nucleotides surrounding the first and optionally the second methionine codon(s) (ATG) at the 5'-end of the sequence or sequence fragment under consideration. The nucleotides following the first ATG must code for at least 35 unambiguous amino acids without any stop codons. If the first ATG has the required amino acids, the second is not examined. If neither meets the requirement, the candidate sequence is not scored. In order to determine whether the EST sequence contains an authentic signal sequence, the DNA and corresponding amino acid sequences surrounding the ATG codon are scored using a set of seven sensors (evaluation parameters) known to be associated with secretion signals.

Use of the above described signal sequence algorithm allowed identification of an EST cluster sequence from the Incyte database, designated 47283 (SEQ ID NO:62). This EST cluster sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) and a proprietary EST DNA database (Lifeseq®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. The homology search was performed using the computer program BLAST or BLAST2 (Altschul *et al.*, *Methods in Enzymology* 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of

Washington, Seattle, Washington. The consensus sequence obtained therefrom was designated DNA56000 (SEQ ID NO:63). In light of the sequence homology between the DNA56000 sequence and the Merck EST clone no. W69233 (SEQ ID NO:61), the Merck clone corresponding to W69233 was purchased and the cDNA insert was obtained and sequenced. The sequence of this cDNA insert is shown in Figure 24 and is herein designated as DNA57694-1341 (SEQ ID NO:64).

Clone DNA57694-1341 (SEQ ID NO:64) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 13-15 and ending at the stop codon at nucleotide positions 310-312 (Figure 24). The predicted polypeptide precursor is 99 amino acids long (Figure 25). The full-length, native sequence PRO826 protein shown in Figure 25 (SEQ ID NO:65) has an estimated molecular weight of about 11,050 daltons and a pI of about 7.47. Analysis of SEQ ID NO:65 evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 22, potential N-myristoylation sites from about amino acid 22 to about amino acid 27 and from about amino acid 90 to about amino acid 95 and an amino acid sequence block having homology to peroxidase from about amino acid 16 to about amino acid 48. Clone DNA57694-1341 has been deposited with ATCC on June 23, 1998 and has been assigned ATCC deposit no. 203017.

M. Isolation of cDNA Clones Encoding Human PRO1068 (UNQ525)

DNA59214-1449 (SEQ ID NO:66) was identified by applying a proprietary signal sequence finding algorithm developed by Genentech, Inc. (South San Francisco, CA) upon ESTs as well as clustered and assembled EST fragments from public (e.g., GenBank) and/or private (LIFESEQ®, Incyte Pharmaceuticals, Inc., Palo Alto, CA) databases. The signal sequence algorithm computes a secretion signal score based on the character of the DNA nucleotides surrounding the first and optionally the second methionine codon(s) (ATG) at the 5'-end of the sequence or sequence fragment under consideration. The nucleotides following the first ATG must code for at least 35 unambiguous amino acids without any stop codons. If the first ATG has the required amino acids, the second is not examined. If neither meets the requirement, the candidate sequence is not scored. In order to determine whether the EST sequence contains an authentic signal sequence, the DNA and corresponding amino acid sequences surrounding the ATG codon are scored using a set of seven sensors (evaluation parameters) known to be associated with secretion signals.

Use of the above described signal sequence algorithm allowed identification of an EST cluster sequence from the LIFESEQ® database, designated Incyte cluster no. 141736 (SEQ ID NO:67). This EST cluster sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. One or more of the ESTs was derived from a human mast cell line from a patient with mast cell leukemia. The homology search was performed using the computer program BLAST or BLAST2 (Altschul *et al.*, *Methods in Enzymology* 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). The consensus sequence obtained therefrom was DNA56094 (SEQ ID NO:68). In light of the sequence homology between the DNA56094

sequence and Incyte EST clone no. 004974 (SEQ ID NO:69), Incyte EST clone no. 004974 was purchased and the cDNA insert was obtained and sequenced. The sequence of this cDNA insert is shown in Figure 26 and is herein designated as DNA592141449 (SEQ ID NO:66).

The full length clone shown in Figure 26 (SEQ ID NO:66) contains a single open reading frame with an apparent translational initiation site at about nucleotide positions 42-44 and ending at the stop codon found at nucleotide positions 14-16. The predicted native sequence PRO1068 polypeptide precursor (SEQ ID NO:70) encoded therefrom is 124 amino acids long, and has a calculated molecular weight of approximately 14,284 Daltons and an estimated pI of approximately 8.14. SEQ ID NO:70 has the following additional features: a signal peptide sequence at about amino acids 1-20, a urotensin II signature sequence at about amino acids 118-123, a cell attachment sequence at about amino acids 64-66, and a potential cAMP- and cGMP-dependent protein kinase phosphorylation site at about amino acids 112-115.

Clone DNA59214-1449 was deposited with the ATCC on July 1, 1998 and is assigned ATCC deposit no. 203046.

N. Isolation of cDNA Clones Encoding Human PRO1132 (UNQ570)

The extracellular domain (ECD) sequences (including the secretion signal sequence, if any) from about 950 known secreted proteins from the Swiss-Prot public database were used to search EST databases. The EST databases included public EST databases (e.g., GenBank), and a proprietary EST database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 [Altschul *et al.*, *Methods in Enzymology*, 266:460-480 (1996)] as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequences. Those comparisons resulting in a BLAST score of 70 (or in some cases, 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington).

A consensus DNA sequence was assembled relative to other EST sequences using phrap. This consensus sequence was designated herein DNA35934 (SEQ ID NO:71).

The DNA35934 consensus sequence (SEQ ID NO:71) served as a template for the creation of oligonucleotides: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a full-length PRO1132 encoding sequence. Forward and reverse PCR primers generally range from 20 to 30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probe sequences are typically 40-55 bp in length. In some cases, additional oligonucleotides are synthesized when the consensus sequence is greater than about 1-1.5kbp. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel *et al.*, *Current Protocols in Molecular Biology*, *supra*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

The forward and reverse PCR primers and hybridization probes employed with the above procedure were:

forward PCR primer: 5'-TCCTGTGACCACCCCTCTAACACC3' (SEQ ID NO:72)

reverse PCR primer: 5'-CTGGAACATCTGCTGCCAGATTC3' (SEQ ID NO:73).

hybridization probe:

(SEQ ID NO:74).

5'-GTCGGATGACAGCAGCAGCCGCATCATCAATGGATCCGACTGCGATATGC-3'

In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the native sequence PRO1132 sequence of Figure 29 (SEQ ID NO:76) using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal kidney. The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes *et al.*, *Science*, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence DNA59767-1489 of Figure 28 (SEQ ID NO:75), and the encoded native sequence PRO1132 sequence of Figure 29 (SEQ ID NO:76). The entire nucleotide sequence of DNA59767-1489 (SEQ ID NO:75) is shown in Figure 28. Clone DNA59767-1489 contains a single open reading frame with an apparent translational initiation site at nucleotide positions about 354-356, and a stop codon at nucleotide positions about 1233-1235 (Fig. 28; SEQ ID NO:75). The predicted polypeptide precursor is 293 amino acids long. The signal peptide is at about amino acid residues 1-22 and the histidine active site is at about amino acid residues 104-109 of Figure 29 (SEQ ID NO:76). Additionally, regions homologous to serine protease have been identified at about residues 241-251, 93-109, 262-285 and 187-193. Clone DNA59767-1489 has been deposited with ATCC (having the actual sequence rather than representations based on sequencing techniques as presented herein) on August 11, 1998 and was assigned ATCC deposit no. 203108. The full-length PRO1132 protein shown in Figure 29 (SEQ ID NO:76) has an estimated molecular weight of about 32,020 daltons and a pI of about 8.7.

Example 2Macular holes

A patient pool with varying stages of macular holes (*i.e.*, 2, 3 or 4) of varying ages are chosen and the presence of macular holes is confirmed. The pool is selected so as to exclude patients with histories of cystoid macula edema, diabetic retinopathy or exudative age-related macular degeneration.

Vision is examined in each subject to determine the best Snellen visual acuity and analyzed by intraocular pressure, fundus photographs, and fluorescein angiography. Each macular hole is graded according to the criteria described by Gass, *Arch. Ophthalmol.* (1988), 106: 629-39. Eyes with Stage 2 holes have a retinal dehiscence along the margin of the areas of deep retinal cyst formation. Stage 3 is characterized by a full-thickness hole with overlying operculum. Macular holes are classified as Stage 4 when a posterior vitreous detachment is present. Treatment is scheduled within 2 weeks of the baseline examination. Under the criteria, patients should be excluded if they had greater than 2+ nuclear sclerotic or posterior subcapsular lens changes. Patients are followed for 6-10 months, with mean follow-up of 8

months. Doses are determined at a level below therapeutic effectiveness, in the middle of the effective range, and at a level well above the minimal effective range.

Eyes are randomly chosen for the indicated level of PRO polypeptide. In addition, some eyes may separately receive 100 µl of intravitreal hyaluronic acid at the time of administration of PRO in an attempt to delay clearance of the PRO polypeptide from the area of the macular hole.

Surgical procedure:

All surgery can be done under local anesthesia with sedation. After the eyes are prepped and draped, a standard three-port vitrectomy may be performed. In eyes with Stage 2 and Stage 3 macular holes, a core vitrectomy is performed. In Stage 4 macular hole, a complete *pars plana* vitrectomy is performed.

If encountered, an epiretinal membrane may be peeled from the surface of the retina and removed from the eye. In other cases, some gelatinous condensation on the inner surface of the retina surrounding the macular hole for about 200-400 µm, with a firm adhesion along the margin of the macular hole. This was carefully dissected where possible, taking care to limit traction on the edges of the macular hole and damage to the nerves.

After allowing for peripheral fluid to drain posteriorly, any fluid which migrates posteriorly is also aspirated. A tapered, bent-tipped cannula is then connected to a 1 cc syringe containing a solution of PRO polypeptide. The reconstituted formulation contains the desired concentration of the test material after dilution. Eyes are randomly assigned a dose of PRO, and about 0.1 cc of the test material solution is gently infused into the macular hole. The same volume of hyaluronic acid may also be administered.

After surgery, the patient should lie in a supine position for the first 24 hours following surgery. Thereafter, each patient should remain in a face-down position as much as possible for a 2 week period.

Patients are examined at 1 day, 2 weeks, 4-6 weeks, and monthly post surgery. Fluorescein angiography is performed at 4 to 6 weeks, 3 months, and 6 months. Best corrected Snellen visual acuity, intraocular pressure, lens status, bubble size, status of macular hole and occurrence of adverse effects are determined at each examination.

Discussion:

The rationale for treatment in this example is to induce the flattening of the edges of the macular hole in order to resolve retinal detachment and thickening surrounding the hole. It has been suggested that a reduction in the traction force which elevates the retina around the hole coupled with and induction of the chorioretinal adhesion along the edge of the hole is necessary for therapeutic effect. Unlike peripheral retinal holes where surgical techniques can be used to reattach the retina and a small area of destruction is not noticeable, macular holes require gentle induction of chorioretinal adhesion to avoid the destruction of adjacent neurosensory tissue and permanent destruction of central vision.

Example 3

Light Induced Photoreceptor Injury

Albino rats (F344 of Sprague-Dawley) of 2-5 months of age are maintained in a cyclic light environment (12 hours on followed by 12 hours off from an in-cage illuminance of less than 25 ft-c) for 9

days or more days before exposure to a constant light source. The constant light source is maintained at an illuminance level of 115-200 ft-c. For example, 2 40 watt white reflector fluorescent bulbs suspended 60 cm above the floor of a transparent polycarbonate cage with stainless steel wire-bar covers.

Two days before the constant light exposure, the rats are anesthetized with a ketamine-xylazine mixture which is administered intravitreally with 1 μ l of the tested factor dissolved in phosphate buffered saline (PBS) at a concentration of 50-1000 ng/ μ l. The injections were made with the insertion of a 32 gauge needle through the sclera, choroid and retina approximately midway between the ora serrata and equator of the eye. The factor-injected animals are compared to either uninjected littermates of those that receive control injections, and to control animals who are not exposed to constant light. Controls should include an injection of PBS alone, or a sham injection (insertion of needle with no injection). In all cases, the injections are made into the superior hemisphere of the eye.

Immediately following the constant light exposure, the rats are killed by any suitable means, *e.g.*, carbon dioxide anesthetization followed by vascular perfusion of mixed aldehydes. The eyes are embedded in epoxy resin and sectioned into 1 μ m thick sections of the entire retina along the vertical meridian of the eye. The degree of light-induced retinal degeneration is then quantified by two methods. The first is through measurement of the outer nuclear layer (ONL) thickness, which is used as an index of photoreceptor cell loss. A mean ONL thickness is obtained from a single section of each animal with the aid of a Bioquant morphometry system. In each of the superior and inferior hemispheres, ONL thickness is measured in 9 sets of 3 measurements each (total of 27 measurements in each hemisphere). Each set is centered on adjacent 440- μ m lengths of retina (the diameter of the microscope field at 400X magnification). The first set of measurements is taken at approximately 440 μ m from the optic nerve head, with subsequent sets taken more peripherally. Within each 440- μ m length of the retina, the 3 measurements are made at defined points separated from one another by 75 μ m. In all, 54 measurements are taken in the two hemispheres which sample representative regions of almost the entire retinal section.

The second method of assessing the degree of photoreceptor rescue is through a subjective evaluation by an examining pathologist on a scale of 0-4+, wherein 4+ is maximal rescue and nearly normal retinal integrity. The degree of photoreceptor rescue in each section, based in comparison to the control eye in the same rat, is scored by four individuals. This method not only takes into account the ONL thickness, but also more subtle degenerative changes to the photoreceptor inner and outer segments, as well as degenerative gradients within the eye.

Discussion:

The intravitreal administration of various factors into the eyes of albino rats can enable the rapid assessment of both the ability of the factors to rescue photoreceptors from degeneration and the side effects, such as incidence of macrophages, associated with each factor. Although the model described herein is the albino rat, the eyes of other albino mammals, such as mice and rabbits, are also useful for this purpose.

Example 4**Retinal Neuron Survival**

Sprague Dawley rat pups at postnatal day 7 (mixed population: glia and retinal neuronal types) are killed by decapitation following CO₂ anesthesia and the eyes are removed under sterile conditions. The neural retina is dissected away from the pigment epithelium and other ocular tissue and then dissociated into a single cell suspension using 0.25% trypsin in Ca²⁺, Mg²⁺-free PBS. The retinas are incubated at 37°C for 7-10 minutes after which the trypsin is inactivated by adding 1 ml soybean trypsin inhibitor. The cells are plated at 100,000 cells per well in 96 well plates in DMEM/F12 supplemented with N2. Cells for all experiments are grown at 37°C in a water saturated atmosphere of 5% CO₂. After 2-3 days in culture, cells are stained with calcein AM or CellTracker Green CMFDA then fixed using 4% paraformaldehyde and stained with DAPI for determination of total cell count. The total cells (fluorescent) are quantified at 20X objective magnification using CCD camera and NIH image software for MacIntosh. Fields in the well are chosen at random. The datapoints are reported as % survival which is the fraction of total number of calcein/CellTracker positive cells after 2-3 days in culture divided by the total number of cells (DAPI-labeled) after 2-3 days in culture.

The effect of various concentration of PRO polypeptide are reported Table 9.

Table 9
Retinal Neuron Survival

Compound	Concentration	% survival
PRO175 (SEQ ID NO:2)	0.01%	0
	0.1%	0
	1.0%	44
PRO220 (SEQ ID NO:8)	0.01%	2.4
	0.01%	4.1
	0.1%	3.0
	0.1%	3.1
	1%	72.4
	1%	42.1
	1%	42.1
PRO216 (SEQ ID NO:10)	0.01%	1.5
	0.1%	1.9
	1%	50.5
	2%	0.8
	2%	2.4
	10%	1.7
	10%	2.1
	20%	30.1
	20%	30.1

	20%	56.1
PRO306 (SEQ ID NO:28)	0.01%	0
	0.01%	2.4
	0.01%	10.8
	0.1%	0
	0.1%	3.6
	0.1%	12.5
	1	35.6
	1	51.7
	1	77.5
PRO322 (SEQ ID NO:42)	2	0
	10	0
	20	52.4
PRO346 (SEQ ID NO:31)	0.1348 nM	0
	1.348 nM	0
	13.48 nM	50.6
PRO840 (SEQ ID NO:54)	0.1509 nM	0
	1.509 nM	0
	15.09 nM	46.7
PRO826 (SEQ ID NO:65)	0.0903 nM	0
	0.903 nM	0
	9.03 nM	49
PRO828 (SEQ ID NO:60)	0.0086 nM	0
	0.086 nM	0
	0.86 nM	55.1
PRO1068 (SEQ ID NO:70)	0.1672 nM	0
	1.672 nM	0
	16.72 nM	44
PRO1132 (SEQ ID NO:76)	0.01%	0.6
	0.1%	3.7
	1%	48.3

Example 5**Rod Photoreceptor Survival**

5 Sprague Dawley rat pups at 7 day postnatal (mixed population: glia and retinal neuronal cell types) are killed by decapitation following CO₂ anesthesia and the eyes are removed under sterile conditions. The neural retina is dissected away from the pigment epithelium and other ocular tissue and then dissociated into

a single cell suspension using 0.25% trypsin in Ca^{2+} , Mg^{2+} -free PBS. The retinas are incubated at 37°C for 7-10 minutes after which the trypsin is inactivated by adding 1 ml soybean trypsin inhibitor. The cells are plated at 100,000 cells per well in 96 well plates in DMEM/F12 supplemented with N2. Cells for all experiments are grown at 37°C in a water saturated atmosphere of 5% CO_2 . After 2-3 days in culture, cells are stained using calcein AM or CellTracker Green CMFDA and then fixed using 4% paraformaldehyde. Rho 4D2 (ascites or IgG 1:100), a monoclonal antibody directed towards the visual pigment rhodopsin is used to detect rod photoreceptor cells by indirect immunofluorescence. The results are reported as % survival: total number of calcein - rhodopsin positive cells at 2-3 days in culture, divided by the total number of rhodopsin positive cells at time 2-3 days in culture. The total cells (fluorescent) are quantified at 20x objective magnification using a CCD camera and NIH image software for MacIntosh. Fields in the well are chosen at random.

The effect of various concentration of PRO are reported in Table 10.

Table 10
Rod Photoreceptor Cell Survival

Compound	Concentration	% survival
PRO175 (SEQ ID NO:2)	0.01%	0
	0.1%	0
	1%	52.9
PRO216 (SEQ ID NO:10)	2%	0
	2%	0
	2%	0
	10%	0
	10%	0
	10%	0
	20%	54
	20%	34.4
	20%	97.1
PRO220 (SEQ ID NO:8)	0.01%	0
	0.1%	0
	2%	0
	10%	0
	20%	66.9
	1%	56.9
PRO243 (SEQ ID NO:15)	0.0188 nM	0
	0.188 nM	0
	1.88 nM	12.7
PRO306 (SEQ ID NO:28)	0.01%	0
	0.1%	0

	1%	35.5
	0.01%	0
	0.1%	0
	1%	58.8
PRO322 (SEQ ID NO:42)	2%	0
	10%	0
	20%	19.4
PRO346 (SEQ ID NO:31)	0.1348 nM	0
	1.348 nM	0
	13.48 nM	30.4
PRO536 (SEQ ID NO:46)	0.01%	0
	0.1%	0.76
	1%	12.9
PRO840 (SEQ ID NO:54)	0.1509 nM	0
	1.509 nM	0
	15.09 nM	43.9
PRO826 (SEQ ID NO:65)	0.0903 nM	0
	0.903 nM	0
	9.03 nM	28.3
PRO828 (SEQ ID NO:60)	0.0086 nM	0
	0.086 nM	0
	0.86 nM	36.8
PRO943 (SEQ ID NO:48)	0.01%	0.76
	0.1%	2.28
	1%	12.9
PRO1068 (SEQ ID NO:70)	0.1672 nM	0
	1.672 nM	0
	16.72 nM	21.7
PRO1132 (SEQ ID NO:76)	0.01%	0
	0.1%	2.28
	1%	48.0

Example 6

Light Ablation Study

Introduction:

As indicated by Reme C.E. *et al.*, *Degen. Dis. Retina*, Ch. 3, Ed. R.E. Anderson *et al.*, Plenum Press, New York (1995), retina degeneration can be induced by exposure to strong light. This light ablation model permits a quantitative comparison of photoreceptor survival promoting activity of a tested substance.

Methods:

Adult female Sprague-Dawley rats are kept in "normal" fluorescent light environment (50 foot candles) for 12 hours on/off until the beginning of the experimental period. Light-induced degeneration is initiated by placing dark adapted (rats kept in 24 hour total darkness). About 5-10 animals in each treatment group are placed into a 5' x 3' chamber illuminated with 490-580 nm (green) light at 300-400 foot candles. Light exposure should be intermittent, 1 hour on, 2 hours off, for a total of eight cycles. Both eyes of each animal should receive 1-2 μ l vitreal injections of test factor two days prior to the light exposure. Test factors employed are 0.5 -1.0 μ g/ μ l of bFGF (SEQ ID NO:67) or PRO polypeptide and controls of phosphate buffered saline with and without bovine serum albumin (0.1%).

Tdt-mediated dUTP nick-end labeling (TUNEL) (Gavrieli, Y *et al.*, *J. Cell Biol.* 119: 493-501 (1992), is performed with modifications using the ApopTag® *In situ* Apoptosis Detection Kit (Oncor®, cat. no. S7110-KIT) on a 4 μ m thick paraffin sections. The DNA strand breaks (fragments) were labeled with fluorescein while intact DNA were labeled with DAPI (4',6-diamidino-2-phenylindole) and visualized with a FITC/DAPI filter on a Vanox AH-3 Olympus microscope.

Results:

The degree of retinal degeneration or survival promoting activity of the tested factors is reported as the thickness of the photoreceptor cell layer or number of TUNEL labeled photoreceptor cell nuclei. Three transverse sections through the central retina (approx. 10 μ m intervals) were used for the analysis. For each section, the entire retinal surface area was digitized using a cooled CCD camera and NIH image software (MacIntosh) to derive the quantitative data.

Example 7

Corneal Pocket Assay

Introduction:

This experiment is intended to determine whether the tested agent is angiogenic in this rodent *in vivo* model. Sample are formulated and pelleted with a delivery vehicle and stability and then transplanted into the cornea and then observed for angiogenic effect. The procedure is adapted from Polverini *et al.*, *Methods in Enzymology* 198: 440-450 (1991).

Methods:

Sprague-Dawley rats (250 g, male) are maintained in plastic carriers under darkened conditions 24 hours prior to treatment and then anesthetized. Each animal's eyes are gently proptosed and secured in place with nontraumatic forceps (*e.g.*, BRI-1725). Using an appropriate blade (*e.g.*, No. 15 blade Bard-Parker), a

1.5 mm incision is made approximately 1 mm from the center of the cornea into the stroma, but not through it. A curved spatula (2 mm wide) is then inserted under the lip of the incision and gently blunt-dissected through the stroma toward the outer canthus of the eye. The final distance between the base of the pocket and the limbus should be at least 1 mm.

5 Pellets are prepared by mixing together tested growth factor (100 ng), sucralfate (50 μ g) and Hydron (e.g., Interferon Sciences, New Brunswick, N.J., Lot # 90005) in a 500:1 ratio of growth factor to sucralfate and Hydron (4 μ l). The sucralfate is present to stabilize the molecule by interacting with the heparin-binding region. The control pellet is composed of Hydron and sucralfate vehicles only. Suggested treatment groups tested are composed of: 1) bovine bFGF (SEQ ID NO:67) (Calbiochem, 10 μ g/50 μ l) PBS + sucralfate (6 animals); 2) sucralfate; and 3) sucralfate mixed with PRO polypeptide.

10 A Hydron pellet (2 x 2 mm) is prepared as described in the previous paragraph and inserted into the base of incision whereupon the pocket should reseal spontaneously. The eyes are coated with artificial tears ointment and then the animals were returned to their plastic carriers, then permitted to awaken and returned to their cages.

15 The assay is terminated on day 5. At time of sacrifice, the animals are perfused with FITC dextran (2 x 10⁶ m.w.) and corneal whole mounts are prepared by careful dissection of the cornea from the eyes, followed by strategic placement of 2-3 cuts to permit permitting the cornea to lie flat, followed by placement under a coverslip. The image is captured through a 1x objective mounted on an inverted fluorescent scope (e.g., Nikon®). Image-Pro® software-edge detection routine can be used to evaluate growth areas.

20

Example 8

Vascular Endothelial Cell Mitogenecity Assay

Introduction:

25 Mitogenic assays on vascular endothelial cells were initially developed in order to monitor the purification of bFGF growth factor. However, they are also a useful measure to determine the presence of mitogenicity in the tested substance.

Materials and Methods:

30 Bovine adrenal cortex-derived capillary endothelial (ACE) cells are established according to known procedures as described by Ferrara *et al.*, *Enzymology* 198, 391-405 (1991). Stock plates of ACE cells are were maintained in 10 cm tissue culture dishes in the presence of low glucose DMEM supplemented with 10% calf serum, 2 mM glutamine and penicillin G (1000 Units/mL) and streptomycin (1000 μ g/mL) and basic FGF (SEQ ID NO:67) at a final concentration of 1 ng/ml and weekly passaged at a split ratio of 1:10. Mitogenic controls are prepared by adding basic FGF at final concentrations of 1 ng/ml and 5 ng/ml and culturing for 5-6 days. ACE cells can be passaged 10-12 times before showing signs of senescence.

35

For each of the test substances, the stock cultures are trypsinized, resuspended in growth media, and seeded at a density of 1.0 x 10⁴ cells/well in 6-well plates (Costar, Cambridge MA), at a plating volume of 2 ml. PRO samples to be tested are added to duplicate or triplicate wells in 10 μ l aliquots, shortly after

plating. After 5 or 6 days, cells are trypsinized and counted in a Coulter Counter (Coulter Electronics, Hialeah, FL).

EXAMPLE 9

Use of PRO as a hybridization probe

The following method describes use of a nucleotide sequence encoding PRO as a hybridization probe.

DNA comprising the coding sequence of full-length or mature PRO as disclosed herein is employed as a probe to screen for homologous DNAs (such as those encoding naturally-occurring variants of PRO) in human tissue cDNA libraries or human tissue genomic libraries.

Hybridization and washing of filters containing either library DNAs is performed under the following high stringency conditions. Hybridization of radiolabeled PRO-derived probe to the filters is performed in a solution of 50% formamide, 5x SSC, 0.1% SDS, 0.1% sodium pyrophosphate, 50 mM sodium phosphate, pH 6.8, 2x Denhardt's solution, and 10% dextran sulfate at 42°C for 20 hours.

Washing of the filters is performed in an aqueous solution of 0.1x SSC and 0.1% SDS at 42°C.

DNAs having a desired sequence identity with the DNA encoding full-length native sequence PRO can then be identified using standard techniques known in the art.

EXAMPLE 10

Expression of PRO in *E. coli*

This example illustrates preparation of an unglycosylated form of PRO by recombinant expression in *E. coli*.

The DNA sequence encoding PRO is initially amplified using selected PCR primers. The primers should contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector. A variety of expression vectors may be employed. An example of a suitable vector is pBR322 (derived from *E. coli*; see Bolivar *et al.*, *Gene*, 2:95 (1977)) which contains genes for ampicillin and tetracycline resistance. The vector is digested with restriction enzyme and dephosphorylated. The PCR amplified sequences are then ligated into the vector. The vector will preferably include sequences which encode for an antibiotic resistance gene, a trp promoter, a polyhis leader (including the first six STII codons, polyhis sequence, and enterokinase cleavage site), the PRO coding region, lambda transcriptional terminator, and an argU gene.

The ligation mixture is then used to transform a selected *E. coli* strain using the methods described in Sambrook *et al.*, *supra*. Transformants are identified by their ability to grow on LB plates and antibiotic resistant colonies are then selected. Plasmid DNA can be isolated and confirmed by restriction analysis and DNA sequencing.

Selected clones can be grown overnight in liquid culture medium such as LB broth supplemented with antibiotics. The overnight culture may subsequently be used to inoculate a larger scale culture. The cells are then grown to a desired optical density, during which the expression promoter is turned on.

After culturing the cells for several more hours, the cells can be harvested by centrifugation. The cell pellet obtained by the centrifugation can be solubilized using various agents known in the art, and the solubilized PRO protein can then be purified using a metal chelating column under conditions that allow tight binding of the protein.

5 PRO may be expressed in *E. coli* in a poly-His tagged form, using the following procedure. The DNA encoding PRO is initially amplified using selected PCR primers. The primers will contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector, and other useful sequences providing for efficient and reliable translation initiation, rapid purification on a metal chelation column, and proteolytic removal with enterokinase. The PCR-
10 amplified, poly-His tagged sequences are then ligated into an expression vector, which is used to transform an *E. coli* host based on strain 52 (W3110 fuhA(tonA) lon galE rpoHts(htpRts) clpP(lacIq). Transformants are first grown in LB containing 50 mg/ml carbenicillin at 30°C with shaking until an O.D.600 of 3-5 is reached. Cultures are then diluted 50-100 fold into CRAP media (prepared by mixing 3.57 g (NH₄)₂SO₄, 0.71 g sodium citrate·2H₂O, 1.07 g KCl, 5.36 g Difco yeast extract, 5.36 g
15 Sheffield hycase SF in 500 mL water, as well as 110 mM MPOS, pH 7.3, 0.55% (w/v) glucose and 7 mM MgSO₄) and grown for approximately 20-30 hours at 30°C with shaking. Samples are removed to verify expression by SDS-PAGE analysis, and the bulk culture is centrifuged to pellet the cells. Cell pellets are frozen until purification and refolding.

E. coli paste from 0.5 to 1 L fermentations (6-10 g pellets) is resuspended in 10 volumes (w/v)
20 in 7 M guanidine, 20 mM Tris, pH 8 buffer. Solid sodium sulfite and sodium tetrathionate is added to make final concentrations of 0.1M and 0.02 M, respectively, and the solution is stirred overnight at 4°C. This step results in a denatured protein with all cysteine residues blocked by sulfitolization. The solution is centrifuged at 40,000 rpm in a Beckman Ultracentrifuge for 30 min. The supernatant is diluted with 3-5 volumes of metal chelate column buffer (6 M guanidine, 20 mM Tris, pH 7.4) and
25 filtered through 0.22 micron filters to clarify. The clarified extract is loaded onto a 5 ml Qiagen Ni-NTA metal chelate column equilibrated in the metal chelate column buffer. The column is washed with additional buffer containing 50 mM imidazole (Calbiochem, Utrol grade), pH 7.4. The protein is eluted with buffer containing 250 mM imidazole. Fractions containing the desired protein are pooled and stored at 4°C. Protein concentration is estimated by its absorbance at 280 nm using the calculated
30 extinction coefficient based on its amino acid sequence.

The proteins are refolded by diluting the sample slowly into freshly prepared refolding buffer consisting of: 20 mM Tris, pH 8.6, 0.3 M NaCl, 2.5 M urea, 5 mM cysteine, 20 mM glycine and 1 mM EDTA. Refolding volumes are chosen so that the final protein concentration is between 50 to 100 micrograms/ml. The refolding solution is stirred gently at 4°C for 12-36 hours. The refolding reaction
35 is quenched by the addition of TFA to a final concentration of 0.4% (pH of approximately 3). Before further purification of the protein, the solution is filtered through a 0.22 micron filter and acetonitrile is added to 2-10% final concentration. The refolded protein is chromatographed on a Poros R1/H reversed phase column using a mobile buffer of 0.1% TFA with elution with a gradient of acetonitrile from 10 to 80%. Aliquots of fractions with A280 absorbance are analyzed on SDS polyacrylamide

gels and fractions containing homogeneous refolded protein are pooled. Generally, the properly refolded species of most proteins are eluted at the lowest concentrations of acetonitrile since those species are the most compact with their hydrophobic interiors shielded from interaction with the reversed phase resin. Aggregated species are usually eluted at higher acetonitrile concentrations. In addition to resolving misfolded forms of proteins from the desired form, the reversed phase step also removes endotoxin from the samples.

Fractions containing the desired folded PRO polypeptide are pooled and the acetonitrile removed using a gentle stream of nitrogen directed at the solution. Proteins are formulated into 20 mM Hepes, pH 6.8 with 0.14 M sodium chloride and 4% mannitol by dialysis or by gel filtration using G25 Superfine (Pharmacia) resins equilibrated in the formulation buffer and sterile filtered.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

EXAMPLE 11

Expression of PRO in mammalian cells

This example illustrates preparation of a potentially glycosylated form of PRO by recombinant expression in mammalian cells.

The vector, pRK5 (see EP 307,247, published March 15, 1989), is employed as the expression vector. Optionally, the PRO DNA is ligated into pRK5 with selected restriction enzymes to allow insertion of the PRO DNA using ligation methods such as described in Sambrook *et al.*, *supra*. The resulting vector is called pRK5-PRO.

In one embodiment, the selected host cells may be 293 cells. Human 293 cells (ATCC CCL 1573) are grown to confluence in tissue culture plates in medium such as DMEM supplemented with fetal calf serum and optionally, nutrient components and/or antibiotics. About 10 µg pRK5-PRO DNA is mixed with about 1 µg DNA encoding the VA RNA gene [Thimmappaya *et al.*, *Cell*, 31:543 (1982)] and dissolved in 500 µl of 1 mM Tris-HCl, 0.1 mM EDTA, 0.227 M CaCl₂. To this mixture is added, dropwise, 500 µl of 50 mM HEPES (pH 7.35), 280 mM NaCl, 1.5 mM NaPO₄, and a precipitate is allowed to form for 10 minutes at 25°C. The precipitate is suspended and added to the 293 cells and allowed to settle for about four hours at 37°C. The culture medium is aspirated off and 2 ml of 20% glycerol in PBS is added for 30 seconds. The 293 cells are then washed with serum free medium, fresh medium is added and the cells are incubated for about 5 days.

Approximately 24 hours after the transfections, the culture medium is removed and replaced with culture medium (alone) or culture medium containing 200 µCi/ml ³⁵S-cysteine and 200 µCi/ml ³⁵S-methionine. After a 12 hour incubation, the conditioned medium is collected, concentrated on a spin filter, and loaded onto a 15% SDS gel. The processed gel may be dried and exposed to film for a selected period of time to reveal the presence of PRO polypeptide. The cultures containing transfected cells may undergo further incubation (in serum free medium) and the medium is tested in selected bioassays.

In an alternative technique, PRO may be introduced into 293 cells transiently using the dextran sulfate method described by Sompayrac *et al.*, *Proc. Natl. Acad. Sci.*, 12:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700 µg pRK5-PRO DNA is added. The cells are first concentrated from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20% glycerol for 90 seconds, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5 µg/ml bovine insulin and 0.1 µg/ml bovine transferrin. After about four days, the conditioned media is centrifuged and filtered to remove cells and debris. The sample containing expressed PRO can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography.

In another embodiment, PRO can be expressed in CHO cells. The pRK5-PRO can be transfected into CHO cells using known reagents such as CaPO₄ or DEAE-dextran. As described above, the cell cultures can be incubated, and the medium replaced with culture medium (alone) or medium containing a radiolabel such as ³⁵S-methionine. After determining the presence of PRO polypeptide, the culture medium may be replaced with serum free medium. Preferably, the cultures are incubated for about 6 days, and then the conditioned medium is harvested. The medium containing the expressed PRO can then be concentrated and purified by any selected method.

Epitope-tagged PRO may also be expressed in host CHO cells. The PRO may be subcloned out of the pRK5 vector. The subclone insert can undergo PCR to fuse in frame with a selected epitope tag such as a poly-his tag into a Baculovirus expression vector. The poly-his tagged PRO insert can then be subcloned into a SV40 driven vector containing a selection marker such as DHFR for selection of stable clones. Finally, the CHO cells can be transfected (as described above) with the SV40 driven vector. Labeling may be performed, as described above, to verify expression. The culture medium containing the expressed poly-His tagged PRO can then be concentrated and purified by any selected method, such as by Ni²⁺-chelate affinity chromatography.

PRO may also be expressed in CHO and/or COS cells by a transient expression procedure or in CHO cells by another stable expression procedure.

Stable expression in CHO cells is performed using the following procedure. The proteins are expressed as an IgG construct (immunoadhesin), in which the coding sequences for the soluble forms (e.g. extracellular domains) of the respective proteins are fused to an IgG1 constant region sequence containing the hinge, CH2 and CH2 domains and/or is a poly-His tagged form.

Following PCR amplification, the respective DNAs are subcloned in a CHO expression vector using standard techniques as described in Ausubel *et al.*, *Current Protocols of Molecular Biology*, Unit 3.16, John Wiley and Sons (1997). CHO expression vectors are constructed to have compatible restriction sites 5' and 3' of the DNA of interest to allow the convenient shuttling of cDNA's. The vector used expression in CHO cells is as described in Lucas *et al.*, *Nucl. Acids Res.* 24:9 (1774-1779 (1996), and uses the SV40 early promoter/enhancer to drive expression of the cDNA of interest and dihydrofolate reductase (DHFR). DHFR expression permits selection for stable maintenance of the plasmid following transfection.

Twelve micrograms of the desired plasmid DNA is introduced into approximately 10 million CHO cells using commercially available transfection reagents Superfect® (Quiagen), Dosper® or Eugene® (Boehringer Mannheim). The cells are grown as described in Lucas *et al.*, *supra*. Approximately 3×10^7 cells are frozen in an ampule for further growth and production as described below.

The ampules containing the plasmid DNA are thawed by placement into water bath and mixed by vortexing. The contents are pipetted into a centrifuge tube containing 10 mLs of media and centrifuged at 1000 rpm for 5 minutes. The supernatant is aspirated and the cells are resuspended in 10 mL of selective media (0.2 µm filtered PS20 with 5% 0.2 µm diafiltered fetal bovine serum). The cells are then aliquoted into a 100 mL spinner containing 90 mL of selective media. After 1-2 days, the cells are transferred into a 250 mL spinner filled with 150 mL selective growth medium and incubated at 37°C. After another 2-3 days, 250 mL, 500 mL and 2000 mL spinners are seeded with 3×10^5 cells/mL. The cell media is exchanged with fresh media by centrifugation and resuspension in production medium. Although any suitable CHO media may be employed, a production medium described in U.S. Patent No. 5,122,469, issued June 16, 1992 may actually be used. A 3L production spinner is seeded at 1.2×10^6 cells/mL. On day 0, the cell number pH is determined. On day 1, the spinner is sampled and sparging with filtered air is commenced. On day 2, the spinner is sampled, the temperature shifted to 33°C, and 30 mL of 500 g/L glucose and 0.6 mL of 10% antifoam (*e.g.*, 35% polydimethylsiloxane emulsion, Dow Corning 365 Medical Grade Emulsion) taken. Throughout the production, the pH is adjusted as necessary to keep it at around 7.2. After 10 days, or until the viability dropped below 70%, the cell culture is harvested by centrifugation and filtering through a 0.22 µm filter. The filtrate was either stored at 4°C or immediately loaded onto columns for purification.

For the poly-His tagged constructs, the proteins are purified using a Ni-NTA column (Qiagen). Before purification, imidazole is added to the conditioned media to a concentration of 5 mM. The conditioned media is pumped onto a 6 ml Ni-NTA column equilibrated in 20 mM Hepes, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 ml/min. at 4°C. After loading, the column is washed with additional equilibration buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein is subsequently desalted into a storage buffer containing 10 mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8, with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.

Immunoadhesin (Fc-containing) constructs are purified from the conditioned media as follows. The conditioned medium is pumped onto a 5 ml Protein A column (Pharmacia) which had been equilibrated in 20 mM Na phosphate buffer, pH 6.8. After loading, the column is washed extensively with equilibration buffer before elution with 100 mM citric acid, pH 3.5. The eluted protein is immediately neutralized by collecting 1 ml fractions into tubes containing 275 µL of 1 M Tris buffer, pH 9. The highly purified protein is subsequently desalted into storage buffer as described above for the poly-His tagged proteins. The homogeneity is assessed by SDS polyacrylamide gels and by N-terminal amino acid sequencing by Edman degradation.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

EXAMPLE 12

Expression of PRO in Yeast

The following method describes recombinant expression of PRO in yeast.

First, yeast expression vectors are constructed for intracellular production or secretion of PRO from the ADH2/GAPDH promoter. DNA encoding PRO and the promoter is inserted into suitable restriction enzyme sites in the selected plasmid to direct intracellular expression of PRO. For secretion, DNA encoding PRO can be cloned into the selected plasmid, together with DNA encoding the ADH2/GAPDH promoter, a native PRO signal peptide or other mammalian signal peptide, or, for example, a yeast alpha-factor or invertase secretory signal/leader sequence, and linker sequences (if needed) for expression of PRO.

Yeast cells, such as yeast strain AB110, can then be transformed with the expression plasmids described above and cultured in selected fermentation media. The transformed yeast supernatants can be analyzed by precipitation with 10% trichloroacetic acid and separation by SDS-PAGE, followed by staining of the gels with Coomassie Blue stain.

Recombinant PRO can subsequently be isolated and purified by removing the yeast cells from the fermentation medium by centrifugation and then concentrating the medium using selected cartridge filters. The concentrate containing PRO may further be purified using selected column chromatography resins.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

EXAMPLE 13

Expression of PRO in Baculovirus-Infected Insect Cells

The following method describes recombinant expression of PRO in Baculovirus-infected insect cells.

The sequence coding for PRO is fused upstream of an epitope tag contained within a baculovirus expression vector. Such epitope tags include poly-his tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pVL1393 (Novagen). Briefly, the sequence encoding PRO or the desired portion of the coding sequence of PRO such as the sequence encoding the extracellular domain of a transmembrane protein or the sequence encoding the mature protein if the protein is extracellular is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector.

Recombinant baculovirus is generated by co-transfecting the above plasmid and BaculoGold™ virus DNA (Pharmingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711)

using lipofectin (commercially available from GIBCO-BRL). After 4 - 5 days of incubation at 28°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression are performed as described by O'Reilley *et al.*, *Baculovirus expression vectors: A Laboratory Manual*, Oxford: Oxford University Press (1994).

5 Expressed poly-his tagged PRO can then be purified, for example, by Ni²⁺-chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Rupert *et al.*, *Nature*, 362:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 mL Hepes, pH 7.9; 12.5 mM MgCl₂; 0.1 mM EDTA; 10% glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50 mM phosphate, 300 mM NaCl, 10% glycerol, pH 7.8) and filtered through a 0.45 µm filter. A Ni²⁺-NTA agarose column (commercially available from Qiagen) is prepared with a bed volume of 5 mL, washed with 25 mL of water and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at 0.5 mL per minute. The column is washed to baseline A₂₈₀ with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50 mM phosphate; 300 mM NaCl, 10% glycerol, pH 6.0), which elutes nonspecifically bound protein. After reaching A₂₈₀ baseline again, the column is developed with a 0 to 500 mM Imidazole gradient in the secondary wash buffer. One mL fractions are collected and analyzed by SDS-PAGE and silver staining or Western blot with Ni²⁺-NTA-conjugated to alkaline phosphatase (Qiagen). Fractions containing the eluted His₁₀-tagged PRO are pooled and dialyzed against loading buffer.

15 Alternatively, purification of the IgG tagged (or Fc tagged) PRO can be performed using known chromatography techniques, including for instance, Protein A or protein G column chromatography.

20 Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

EXAMPLE 14

Preparation of Antibodies that Bind PRO

30 This example illustrates preparation of monoclonal antibodies which can specifically bind PRO.

 Techniques for producing the monoclonal antibodies are known in the art and are described, for instance, in Goding, *supra*. Immunogens that may be employed include purified PRO, fusion proteins containing PRO, and cells expressing recombinant PRO on the cell surface. Selection of the immunogen can be made by the skilled artisan without undue experimentation.

35 Mice, such as Balb/c, are immunized with the PRO immunogen emulsified in complete Freund's adjuvant and injected subcutaneously or intraperitoneally in an amount from 1-100 micrograms. Alternatively, the immunogen is emulsified in MPL-TDM adjuvant (Ribi Immunochemical Research, Hamilton, MT) and injected into the animal's hind foot pads. The immunized mice are then boosted 10 to 12 days later with additional immunogen emulsified in the

selected adjuvant. Thereafter, for several weeks, the mice may also be boosted with additional immunization injections. Serum samples may be periodically obtained from the mice by retro-orbital bleeding for testing in ELISA assays to detect anti-PRO antibodies.

5 After a suitable antibody titer has been detected, the animals "positive" for antibodies can be injected with a final intravenous injection of PRO. Three to four days later, the mice are sacrificed and the spleen cells are harvested. The spleen cells are then fused (using 35% polyethylene glycol) to a selected murine myeloma cell line such as P3X63AgU.1, available from ATCC, No. CRL 1597. The fusions generate hybridoma cells which can then be plated in 96 well tissue culture plates containing HAT (hypoxanthine, aminopterin, and thymidine) medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

10 The hybridoma cells will be screened in an ELISA for reactivity against PRO. Determination of "positive" hybridoma cells secreting the desired monoclonal antibodies against PRO is within the skill in the art.

15 The positive hybridoma cells can be injected intraperitoneally into syngeneic Balb/c mice to produce ascites containing the anti-PRO monoclonal antibodies. Alternatively, the hybridoma cells can be grown in tissue culture flasks or roller bottles. Purification of the monoclonal antibodies produced in the ascites can be accomplished using ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can be employed.

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EXAMPLE 15

Purification of PRO Polypeptides Using Specific Antibodies

Native or recombinant PRO polypeptides may be purified by a variety of standard techniques in the art of protein purification. For example, pro-PRO polypeptide, mature PRO polypeptide, or pre-PRO polypeptide is purified by immunoaffinity chromatography using antibodies specific for the PRO polypeptide of interest. In general, an immunoaffinity column is constructed by covalently coupling the anti-PRO polypeptide antibody to an activated chromatographic resin.

25 Polyclonal immunoglobulins are prepared from immune sera either by precipitation with ammonium sulfate or by purification on immobilized Protein A (Pharmacia LKB Biotechnology, Piscataway, N.J.). Likewise, monoclonal antibodies are prepared from mouse ascites fluid by ammonium sulfate precipitation or chromatography on immobilized Protein A. Partially purified immunoglobulin is covalently attached to a chromatographic resin such as CnBr-activated SEPHAROSE™ (Pharmacia LKB Biotechnology). The antibody is coupled to the resin, the resin is blocked, and the derivative resin is washed according to the manufacturer's instructions.

30 Such an immunoaffinity column is utilized in the purification of PRO polypeptide by preparing a fraction from cells containing PRO polypeptide in a soluble form. This preparation is derived by solubilization of the whole cell or of a subcellular fraction obtained via differential centrifugation by the addition of detergent or by other methods well known in the art. Alternatively,

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soluble PRO polypeptide containing a signal sequence may be secreted in useful quantity into the medium in which the cells are grown.

A soluble PRO polypeptide-containing preparation is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of PRO polypeptide (e.g., high ionic strength buffers in the presence of detergent). Then, the column is eluted under conditions that disrupt antibody/PRO polypeptide binding (e.g., a low pH buffer such as approximately pH 2-3, or a high concentration of a chaotrope such as urea or thiocyanate ion), and PRO polypeptide is collected.

EXAMPLE 16

Drug Screening

This invention is particularly useful for screening compounds by using PRO polypeptides or binding fragment thereof in any of a variety of drug screening techniques. The PRO polypeptide or fragment employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the PRO polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, the formation of complexes between PRO polypeptide or a fragment and the agent being tested. Alternatively, one can examine the diminution in complex formation between the PRO polypeptide and its target cell or target receptors caused by the agent being tested.

Thus, the present invention provides methods of screening for drugs or any other agents which can affect a PRO polypeptide-associated disease or disorder. These methods comprise contacting such an agent with an PRO polypeptide or fragment thereof and assaying (i) for the presence of a complex between the agent and the PRO polypeptide or fragment, or (ii) for the presence of a complex between the PRO polypeptide or fragment and the cell, by methods well known in the art. In such competitive binding assays, the PRO polypeptide or fragment is typically labeled. After suitable incubation, free PRO polypeptide or fragment is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of the particular agent to bind to PRO polypeptide or to interfere with the PRO polypeptide/cell complex.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to a polypeptide and is described in detail in WO 84/03564, published on September 13, 1984. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. As applied to a PRO polypeptide, the peptide test compounds are reacted with PRO polypeptide and washed. Bound PRO polypeptide is detected by methods well known in the art. Purified PRO polypeptide can also be coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies can be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding PRO polypeptide specifically compete with a test compound for binding to PRO polypeptide or fragments thereof. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with PRO polypeptide.

EXAMPLE 17

Rational Drug Design

The goal of rational drug design is to produce structural analogs of biologically active polypeptide of interest (*i.e.*, a PRO polypeptide) or of small molecules with which they interact, *e.g.*, agonists, antagonists, or inhibitors. Any of these examples can be used to fashion drugs which are more active or stable forms of the PRO polypeptide or which enhance or interfere with the function of the PRO polypeptide *in vivo* (*c.f.*, Hodgson, *Bio/Technology*, 9: 19-21 (1991)).

In one approach, the three-dimensional structure of the PRO polypeptide, or of an PRO polypeptide-inhibitor complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the PRO polypeptide must be ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, useful information regarding the structure of the PRO polypeptide may be gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design analogous PRO polypeptide-like molecules or to identify efficient inhibitors. Useful examples of rational drug design may include molecules which have improved activity or stability as shown by Braxton and Wells, *Biochemistry*, 31:7796-7801 (1992) or which act as inhibitors, agonists, or antagonists of native peptides as shown by Athauda *et al.*, *J. Biochem.*, 113:742-746 (1993).

It is also possible to isolate a target-specific antibody, selected by functional assay, as described above, and then to solve its crystal structure. This approach, in principle, yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced peptides. The isolated peptides would then act as the pharmacore.

By virtue of the present invention, sufficient amounts of the PRO polypeptide may be made available to perform such analytical studies as X-ray crystallography. In addition, knowledge of the PRO polypeptide amino acid sequence provided herein will provide guidance to those employing computer modeling techniques in place of or in addition to x-ray crystallography.

Deposit of Material

The following materials have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, USA (ATCC).

	<u>Material</u>	<u>ATCC Deposit No.</u>	<u>Deposit Date</u>
5	DNA19355-1150	209466	November 18, 1997
	DNA32298-1132	209257	September 16, 1997
	DNA33087-1158	209381	September 16, 1997
	DNA35917-1207	209508	December 3, 1997
	DNA39984-1221	209435	November 7, 1997
10	DNA44167-1243	209434	November 7, 1997
	DNA48336-1309	209669	March 11, 1998
	DNA49142-1430	203002	June 23, 1998
	DNA52192-1369	203042	July 1, 1998
	DNA53987-1438	209858	May 12, 1998
15	DNA57037-1444	209903	May 27, 1998
	DNA57694-1341	203017	June 23, 1998
	DNA59214-1449	203046	July 1, 1998
	DNA59767-1489	203108	August 11, 1998

20 These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures the maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by the ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and the ATCC, which assures permanent and

25 unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pretinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner's rules pursuant thereto (including 37 C.F.R. § 1.14 with particular reference to 886 OG 638).

30 The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

35 The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present inventions are not to be limited in scope by the construct deposited., since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate

to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method of promoting the survival of retinal cells, said method comprising contacting said retinal cells with an effective amount of an active PRO175, PRO220, PRO216, PRO243, PRO306, PRO346, PRO322, PRO536, PRO943, PRO840, PRO828, PRO826, PRO1068 or PRO1132 polypeptide.

2. The method of Claim 1, wherein the active PRO175, PRO220, PRO216, PRO243, PRO306, PRO346, PRO322, PRO536, PRO943, PRO840, PRO828, PRO826, PRO1068 or PRO1132 polypeptide has at least 80% amino acid sequence identity to the sequence of amino acid residues from about: (a) 1 or about 45 to 177 of Figure 2 (SEQ ID NO:2); (b) 1 or about 23 to 708 of Figure 4 (SEQ ID NO:8); (c) 1 or about 21 to 421 of Figure 6 (SEQ ID NO:10); (d) 1 or about 24 to 954 of Figure 9 (SEQ ID NO:15); (e) 1 or about 23 to 424 of Figure 11 (SEQ ID NO:18); (f) 1 or about 19 to 450 of Figure 13 (SEQ ID NO:21); (g) 1 or about 24 to 260 of Figure 15 (SEQ ID NO:32); (h) 1 or about 26 to 313 of Figure 17 (SEQ ID NO:36); (i) 1 or about 18 to 504 of Figure 19 (SEQ ID NO:38); (j) 1 or about 47 to 437 of Figure 21 (SEQ ID NO:44); (k) 1 or about 22 to 187 of Figure 23 (SEQ ID NO:50); (l) 1 or about 23 to 99 of Figure 25 (SEQ ID NO:55); (m) 1 or about 21 to 124 of Figure 27 (SEQ ID NO:60) or (n) 1 or about 23 to 293 of Figure 29 (SEQ ID NO:66), respectively.

3. The method of Claim 1, wherein the active PRO175, PRO220, PRO216, PRO243, PRO306, PRO346, PRO322, PRO536, PRO943, PRO840, PRO828, PRO826, PRO1068 or PRO1132 polypeptide comprises amino acids: (a) 1 or about 45 to 177 of Figure 2 (SEQ ID NO:2); (b) 1 or about 23 to 708 of Figure 4 (SEQ ID NO:8); (c) 1 or about 21 to 421 of Figure 6 (SEQ ID NO:10); (d) 1 or about 24 to 954 of Figure 9 (SEQ ID NO:15); (e) 1 or about 23 to 424 of Figure 11 (SEQ ID NO:18); (f) 1 or about 19 to 450 of Figure 13 (SEQ ID NO:21); (g) 1 or about 24 to 260 of Figure 15 (SEQ ID NO:32); (h) 1 or about 26 to 313 of Figure 17 (SEQ ID NO:36); (i) 1 or about 18 to 504 of Figure 19 (SEQ ID NO:38); (j) 1 or about 47 to 437 of Figure 21 (SEQ ID NO:44); (k) 1 or about 22 to 187 of Figure 23 (SEQ ID NO:50); (l) 1 or about 23 to 99 of Figure 25 (SEQ ID NO:55); (m) 1 or about 21 to 124 of Figure 27 (SEQ ID NO:60) or (n) 1 or about 23 to 293 of Figure 29 (SEQ ID NO:66), respectively.

4. The method of Claim 1, wherein the active PRO175, PRO220, PRO216, PRO243, PRO306, PRO346, PRO322, PRO536, PRO943, PRO840, PRO828, PRO826, PRO1068 or PRO1132 polypeptide consists of amino acids: (a) 1 or about 45 to 177 of Figure 2 (SEQ ID NO:2); (b) 1 or about 23 to 708 of Figure 4 (SEQ ID NO:8); (c) 1 or about 21 to 421 of Figure 6 (SEQ ID NO:10); (d) 1 or about 24 to 954 of Figure 9 (SEQ ID NO:15); (e) 1 or about 23 to 424 of Figure 11 (SEQ ID NO:18); (f) 1 or about 19 to 450 of Figure 13 (SEQ ID NO:21); (g) 1 or about 24 to 260 of Figure 15 (SEQ ID NO:32); (h) 1 or about 26 to 313 of Figure 17 (SEQ ID NO:36); (i) 1 or about 18 to 504 of Figure 19 (SEQ ID NO:38); (j) 1 or about 47 to 437 of Figure 21 (SEQ ID NO:44); (k) 1 or about 22 to 187 of Figure 23 (SEQ ID NO:50); (l) 1 or about 23 to 99 of Figure 25 (SEQ ID NO:55); (m) 1 or about 21 to 124 of Figure 27 (SEQ ID NO:60) or (n) 1 or about 23 to 293 of Figure 29 (SEQ ID NO:66), respectively.

5. The method of Claim 1, wherein the active PRO175, PRO220, PRO216, PRO243, PRO306, PRO346, PRO322, PRO536, PRO943, PRO840, PRO828, PRO826, PRO1068 or PRO1132 polypeptide has at least about 80% amino acid sequence identity to the polypeptide encoded by the cDNA insert of the vector deposited with the ATCC under ATCC deposit number 209466, 209257, 209381, 209508, 209435, 209434, 209669, 203002, 203042, 209858, 209903, 203017, 203046 or 203108, respectively.
6. The method of Claim 1, wherein the active PRO175, PRO220, PRO216, PRO243, PRO306, PRO346, PRO322, PRO536, PRO943, PRO840, PRO828, PRO826, PRO1068 or PRO1132 polypeptide comprises the amino acid sequence encoded by the cDNA insert of the vector deposited with the ATCC under ATCC deposit number 209466, 209257, 209381, 209508, 209435, 209434, 209669, 203002, 203042, 209858, 209903, 203017, 203046 or 203108, respectively.
7. The method of Claim 1, wherein the active PRO175, PRO220, PRO216, PRO243, PRO306, PRO346, PRO322, PRO536, PRO943, PRO840, PRO828, PRO826, PRO1068 or PRO1132 polypeptide consists of the amino acid sequence encoded by the cDNA insert of the vector deposited with the ATCC under ATCC deposit number 209466, 209257, 209381, 209508, 209435, 209434, 209669, 203002, 203042, 209858, 209903, 203017, 203046 or 203108, respectively.
8. The method of Claim 1, wherein said contacting is performed *in vivo* and wherein said retinal cells are contacted with a therapeutically effective amount of said PRO175, PRO220, PRO216, PRO243, PRO306, PRO346, PRO322, PRO536, PRO943, PRO840, PRO828, PRO826, PRO1068 or PRO1132 polypeptide.
9. The method of Claim 1, wherein said retinal cells are retinal neurons, rod photoreceptors or supportive cells.
10. The method of Claim 9, wherein said retinal neurons are retinal ganglion cells, displaced retinal ganglion cells, amacrine cells, displaced amacrine cells, horizontal neurons or bipolar neurons.
11. The method of Claim 9, wherein said supportive cells are Müller cells or pigment epithelial cells.
12. The method of Claim 1, wherein said contacting is intraocular.
13. The method of Claim 1, wherein the polypeptide is administered into the vitreous or into the subretinal space.
14. The method of Claim 1, wherein said contacting is intravitreal.

15. The method of Claim 1, wherein said contacting is performed by means of an implant.
16. The method of Claim 1, wherein the method further comprises contacting said retinal cells with an effective amount of an active neuronal survival agent.
17. The method of claim 16 wherein the neuronal survival agent is selected from the group consisting of: Vitamin A, an antioxidant, a calcium overload blockers, aFGF, bFGF, TGB- β , BDNF, NT-4, IGF-I, CNTF, IL-1, NT-3, IGF-II, TNF- α , TNF- β and NGF.
18. A method of delaying or preventing retinal cell injury or death, said method comprising contacting said retinal cells with an effective amount of an active PRO175, PRO220, PRO216, PRO243, PRO306, PRO346, PRO322, PRO536, PRO943, PRO840, PRO828, PRO826, PRO1068 or PRO1132 polypeptide.
19. The method of Claim 18, wherein said contacting is performed *in vivo* and wherein said retinal cells are contacted with a therapeutically effective amount of said PRO175, PRO220, PRO216, PRO243, PRO306, PRO346, PRO322, PRO536, PRO943, PRO840, PRO828, PRO826, PRO1068 or PRO1132 polypeptide.
20. The method of Claim 18, wherein said retinal cells are retinal neurons, rod photoreceptors or supportive cells.
21. The method of Claim 18, wherein said contacting is intraocular.
22. The method of Claim 18, wherein the polypeptide is administered into the vitreous or into the subretinal space.
23. The method of Claim 18, wherein said contacting is performed by means of an implant.
24. The method of Claim 18, wherein said retinal cell injury or death is caused by an ocular disease, retinal injury or environmental trauma.
25. The method of Claim 18, wherein the method further comprises contacting said retinal cells with a therapeutically effective amount of an active neuronal survival agent.
26. The method of Claim 25 wherein the neuronal survival agent is selected from the group consisting of: Vitamin A, an antioxidant, a calcium overload blockers, aFGF, bFGF, TGB- β , BDNF, NT-4, IGF-I, CNTF, IL-1, NT-3, IGF-II, TNF- α , TNF- β and NGF.

27. The method of Claim 24, wherein said ocular disease is or is associated with retinitis pigmentosa, macular degeneration, retinal detachment, a retinal tear, retinopathy, a retinal degenerative disease, a macular hole, degenerative myopia, acute retinal necrosis syndrome, a traumatic chorioretinopathy or contusion, Purtscher's retinopathy, edema, an ischemic conditions, central or branch retinal vision occlusion, a collagen vascular disease, thrombocytopenic purpura, uveitis, retinal vasculitis, an occlusion associated with Eales disease or systemic lupus erythematosus.

28. The method of Claim 18 which does not substantially cause angiogenesis or mitogenesis.

29. An article of manufacture, comprising:

- (a) a container;
- (b) a label on said container; and
- (c) a composition contained within said container;

wherein the composition comprises an active agent effective for promoting the survival of retinal cells, the label on said container indicates that the composition is useful for promoting the survival of retinal cells, and said active agent is an active PRO175, PRO220, PRO216, PRO243, PRO306, PRO346, PRO322, PRO536, PRO943, PRO840, PRO828, PRO826, PRO1068 or PRO1132 polypeptide.

30. The article of manufacture of Claim 29 further comprising instructions for administering the PRO175, PRO220, PRO216, PRO243, PRO306, PRO346, PRO322, PRO536, PRO943, PRO840, PRO828, PRO826, PRO1068 or PRO1132 polypeptide to a mammal.

31. An article of manufacture, comprising:

- (a) a container;
- (b) a label on said container; and
- (c) a composition contained within said container;

wherein the composition comprises an active agent effective for delaying or preventing retinal cell injury or death, the label on said container indicates that the composition is useful for delaying or preventing retinal cell injury or death, and said active agent is an active PRO175, PRO220, PRO216, PRO243, PRO306, PRO346, PRO322, PRO536, PRO943, PRO840, PRO828, PRO826, PRO1068 or PRO1132 polypeptide.

32. The article of manufacture of Claim 31 further comprising instructions for administering the PRO175, PRO220, PRO216, PRO243, PRO306, PRO346, PRO322, PRO536, PRO943, PRO840, PRO828, PRO826, PRO1068 or PRO1132 polypeptide to a mammal.

33. Isolated nucleic acid having at least 80% nucleic acid sequence identity to a nucleotide sequence that encodes an amino acid sequence selected from the group consisting of the amino acid sequence shown in Figure 1 (SEQ ID NO:1), Figure 3 (SEQ ID NO:7), Figure 5 (SEQ ID NO:9), Figure 8 (SEQ ID NO:14), Figure 10 (SEQ ID NO:27), Figure 12 (SEQ ID NO:30), Figure 14 (SEQ ID NO:41),

Figure 16 (SEQ ID NO:43), Figure 18 (SEQ ID NO:49), Figure 20 (SEQ ID NO:53), Figure 22 (SEQ ID NO:59), Figure 24 (SEQ ID NO:64), Figure 26 (SEQ ID NO:66) or Figure 28 (SEQ ID NO:75).

34. Isolated nucleic acid having at least 80% nucleic acid sequence identity to a nucleotide sequence selected from the group consisting of the nucleotide sequence shown in Figure 1 (SEQ ID NO:1), Figure 3 (SEQ ID NO:7), Figure 5 (SEQ ID NO:9), Figure 8 (SEQ ID NO:14), Figure 10 (SEQ ID NO:27), Figure 12 (SEQ ID NO:30), Figure 14 (SEQ ID NO:41), Figure 16 (SEQ ID NO:43), Figure 18 (SEQ ID NO:49), Figure 20 (SEQ ID NO:53), Figure 22 (SEQ ID NO:59), Figure 24 (SEQ ID NO:64), Figure 26 (SEQ ID NO:66) or Figure 28 (SEQ ID NO:75).

35. Isolated nucleic acid having at least 80% nucleic acid sequence identity to a nucleotide sequence selected from the group consisting of the full-length coding sequence of the nucleotide sequence shown in Figure 1 (SEQ ID NO:1), Figure 3 (SEQ ID NO:7), Figure 5 (SEQ ID NO:9), Figure 8 (SEQ ID NO:14), Figure 10 (SEQ ID NO:27), Figure 12 (SEQ ID NO:30), Figure 14 (SEQ ID NO:41), Figure 16 (SEQ ID NO:43), Figure 18 (SEQ ID NO:49), Figure 20 (SEQ ID NO:53), Figure 22 (SEQ ID NO:59), Figure 24 (SEQ ID NO:64), Figure 26 (SEQ ID NO:66) or Figure 28 (SEQ ID NO:75).

36. Isolated nucleic acid having at least 80% nucleic acid sequence identity to the full-length coding sequence of the DNA deposited under ATCC accession number 209466, 209257, 209381, 209508, 209435, 209434, 209669, 203002, 203042, 209858, 209903, 203017, 203246 or 203108.

37. A vector comprising the nucleic acid of any one of Claims 33 to 36.

38. The vector of Claim 37 operably linked to control sequences recognized by a host cell transformed with the vector.

39. A host cell comprising the vector of Claim 37.

40. The host cell of Claim 39 wherein said cell is a CHO cell.

41. The host cell of Claim 39, wherein said cell is an *E. coli*.

42. The host cell of Claim 39, wherein said cell is a yeast cell.

43. A process for producing a PRO175, PRO220, PRO216, PRO243, PRO306, PRO346, PRO322, PRO536, PRO943, PRO840, PRO828, PRO826, PRO1068 or PRO1132 polypeptide comprising culturing the host cell of Claim 39 under conditions suitable for expression of said polypeptide and recovering said polypeptide from the cell culture.

44. An isolated polypeptide having at least 80% amino acid sequence identity to an amino acid sequence selected from the group consisting of the amino acid sequence shown in Figure 2 (SEQ ID

NO:2), Figure 4 (SEQ ID NO:8), Figure 6 (SEQ ID NO:10), Figure 9 (SEQ ID NO:15), Figure 11 (SEQ ID NO:28), Figure 13 (SEQ ID NO:31), Figure 15 (SEQ ID NO:42), Figure 17 (SEQ ID NO:46), Figure 19 (SEQ ID NO:48), Figure 21 (SEQ ID NO:54), Figure 23 (SEQ ID NO:60), Figure 25 (SEQ ID NO:65), Figure 27 (SEQ ID NO:70) or Figure 29 (SEQ ID NO:76).

45. An isolated polypeptide scoring at least 80% positives when compared to an amino acid sequence selected from the group consisting of the amino acid sequence shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:8), Figure 6 (SEQ ID NO:10), Figure 9 (SEQ ID NO:15), Figure 11 (SEQ ID NO:28), Figure 13 (SEQ ID NO:31), Figure 15 (SEQ ID NO:42), Figure 17 (SEQ ID NO:46), Figure 19 (SEQ ID NO:48), Figure 21 (SEQ ID NO:54), Figure 23 (SEQ ID NO:60), Figure 25 (SEQ ID NO:65), Figure 27 (SEQ ID NO:70) or Figure 29 (SEQ ID NO:76).

46. An isolated polypeptide having at least 80% amino acid sequence identity to an amino acid sequence encoded by the full-length coding sequence of the DNA deposited under ATCC accession number 209466, 209257, 209381, 209508, 209435, 209434, 209669, 203002, 203042, 209858, 209903, 203017, 203246 or 203108.

47. A chimeric molecule comprising a polypeptide according to any one of Claims 44 to 46 fused to a heterologous amino acid sequence.

48. The chimeric molecule of Claim 47, wherein said heterologous amino acid sequence is an epitope tag sequence.

49. The chimeric molecule of Claim 47, wherein said heterologous amino acid sequence is a Fc region of an immunoglobulin.

50. An antibody which specifically binds to a polypeptide according to any one of Claims 44 to 46.

51. The antibody of Claim 50, wherein said antibody is a monoclonal antibody, a humanized antibody or a single-chain antibody.

52. Isolated nucleic acid having at least 80% nucleic acid sequence identity to:

(a) a nucleotide sequence encoding the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:8), Figure 6 (SEQ ID NO:10), Figure 9 (SEQ ID NO:15), Figure 11 (SEQ ID NO:28), Figure 13 (SEQ ID NO:31), Figure 15 (SEQ ID NO:42), Figure 17 (SEQ ID NO:46), Figure 19 (SEQ ID NO:48), Figure 21 (SEQ ID NO:54), Figure 23 (SEQ ID NO:60), Figure 25 (SEQ ID NO:65), Figure 27 (SEQ ID NO:70) or Figure 29 (SEQ ID NO:76) lacking its associated signal peptide;

(b) a nucleotide sequence encoding an extracellular domain of the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:8), Figure 6 (SEQ ID NO:10), Figure 9 (SEQ ID NO:15,

Figure 11 (SEQ ID NO:28), Figure 13 (SEQ ID NO:31), Figure 15 (SEQ ID NO:42), Figure 17 (SEQ ID NO:46), Figure 19 (SEQ ID NO:48), Figure 21 (SEQ ID NO:54), Figure 23 (SEQ ID NO:60), Figure 25 (SEQ ID NO:65), Figure 27 (SEQ ID NO:70) or Figure 29 (SEQ ID NO:76) with its associated signal peptide; or

(c) a nucleotide sequence encoding an extracellular domain of the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:8), Figure 6 (SEQ ID NO:10), Figure 9 (SEQ ID NO:15), Figure 11 (SEQ ID NO:28), Figure 13 (SEQ ID NO:31), Figure 15 (SEQ ID NO:42), Figure 17 (SEQ ID NO:46), Figure 19 (SEQ ID NO:48), Figure 21 (SEQ ID NO:54), Figure 23 (SEQ ID NO:60), Figure 25 (SEQ ID NO:65), Figure 27 (SEQ ID NO:70) or Figure 29 (SEQ ID NO:76) lacking its associated signal peptide.

53. An isolated polypeptide having at least 80% amino acid sequence identity to:

(a) the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:8), Figure 6 (SEQ ID NO:10), Figure 9 (SEQ ID NO:15), Figure 11 (SEQ ID NO:28), Figure 13 (SEQ ID NO:31), Figure 15 (SEQ ID NO:42), Figure 17 (SEQ ID NO:46), Figure 19 (SEQ ID NO:48), Figure 21 (SEQ ID NO:54), Figure 23 (SEQ ID NO:60), Figure 25 (SEQ ID NO:65), Figure 27 (SEQ ID NO:70) or Figure 29 (SEQ ID NO:76) lacking its associated signal peptide;

(b) an extracellular domain of the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:8), Figure 6 (SEQ ID NO:10), Figure 9 (SEQ ID NO:15), Figure 11 (SEQ ID NO:28), Figure 13 (SEQ ID NO:31), Figure 15 (SEQ ID NO:42), Figure 17 (SEQ ID NO:46), Figure 19 (SEQ ID NO:48), Figure 21 (SEQ ID NO:54), Figure 23 (SEQ ID NO:60), Figure 25 (SEQ ID NO:65), Figure 27 (SEQ ID NO:70) or Figure 29 (SEQ ID NO:76), with its associated signal peptide; or

(c) an extracellular domain of the polypeptide shown in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:8), Figure 6 (SEQ ID NO:10), Figure 9 (SEQ ID NO:15), Figure 11 (SEQ ID NO:28), Figure 13 (SEQ ID NO:31), Figure 15 (SEQ ID NO:42), Figure 17 (SEQ ID NO:46), Figure 19 (SEQ ID NO:48), Figure 21 (SEQ ID NO:54), Figure 23 (SEQ ID NO:60), Figure 25 (SEQ ID NO:65), Figure 27 (SEQ ID NO:70) or Figure 29 (SEQ ID NO:76), lacking its associated signal peptide.

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FIGURE 1

CAGCTCTCATTCTCCAAAA
ATGTGTTTGAGCCACTTGGAATAATGCCTTTAAGCCATTCAAGAAGCTCAAGGAGCTCAGAGATCATCC
TGGAAAGCTGTGGCTCTTTTGCTCAATAGTTATGTTGCTATTTCTTTGCTCCTTCAGTTGGCTAATCTTT
ATTTTCTCCAATTAGAGACTGCTAAGGAGCCCTGTATGGCTAAGTTTGGACCATTACCCTCAAAATGG
CAAATGGCATCTTCTGAACCTCCTTGCCTGAATAAGGTGCTGACTGGAAGCTGGAGATACTTCAGAAAT
GGCTTATATTTAATTTATGGCCAAGTGGCTCCCAATGCAAACTACAATGATGTAGCTCCTTTTGAGGTG
CGGCTGTATAAAAAACAAAGACATGATACAACTCTAACAAACAAATCTAAAATCCAAATGTAGGAGGG
ACTTATGAATTGCATGTTGGGGACACCATAGACTTGATATTCAACTCTGAGCATCAGGTTCTAAAAAAT
AATACATACTGGGGTATCATTCTACTAGCAAATCCCCAATTCATCTCCTAGAGACTTGATTTGATCTCC
TCATTCCCTTCAGCACATGTAGAGGTGCCAGTGGGTGGATTGGAGGGAGAAGATATTCAATTTCTAGAG
TTTGTCTGTCTACAAAAATCAACACAAACAGAAGCTCCTCTGCACGTGAATTTTCATCTATCATGCCTAT
CTGAAAGAGACTCAGGGGAAGAGCCAAAGACTTTTGGTTGGATCTGCAGAAATACTTCATTAATCCATG
ATAAAACAAATATGGATGACAGAGGACATGTGCTTTTCAAAGAATCTTTATCTAATTCCTGAATTCATG
AGTGGAAAAATGGAGTTCATTCCCATGGAAGATTTACCTGGTATGCAAAAAGGATCTGGGGCAGTAGC
CTGGCTTTGTCTCATATTCTTGGGCTGCTGTAATTCATTCTTCTCATACTCCCATCTTCTGAGACCCT
CCCAATAAAAAGTAGACTGATAGGATGGCCACAGATATGCCTACCATAACCTACTTTAGATATGGTGGT
GTTAGAAGATAAAGAACAATCTGAGAAGTATTGGAATAGAGGTACAAGTGGCATAAAATGGAATGTACG
CTATCTGGAATTTCTCTTGGTTTTATCTTCTCAGGATGCAGGGTGCTTTAAAAAGCCTTATCAAAGG
AGTCATTCCGAACCCTCACGTAGAGCTTTGTGAGACCTTACTGTTGGTGTGTGTCTAAACATTGCTA
ATTGTAAAGAAAGAGTAACCATTAGTAATCATTAGGTTTAACCCAGAAATGGTATTATCATTACTGGAT
TATGTCATGTAATGATTTAGTATTTTGTAGCTAGCTTTCCACAGTTTGCAAAGTGCTTTCGTAAACAGT
TAGCAATTCTATGAAGTTAATTGGGCAGGCATTTGGGGGAAAAATTTAGTGATGAGAATGTGATAGCAT
AGCATAGCCAACCTTTCTCAACTCATAGGACAAGTGACTACAAGAGGCAATGGGTAGTCCCCTGCATTG
CACTGTCTCAGCTTTAGAATTGTTATTTCTGCTATCGTGTTATAAGACTCTAAAACCTTAGCGAATTCAC
TTTTTCAGGAAGCATATTCCCCTTTAGCCCAAGGTGAGCAGAGTGAAGCTACAACAGATCTTTCCTTTAC
CAGCACACTTTTTTTTTTTTTTCTGCTGAATCAGGGAGATCCAGGATGCTGTTTCAGGCCAAATCCCA
ACCAAATCCCCTTTTCACTTTGCAGGGCCCATCTTAGTCAAATGTGCTAACTTCTAAAATAATAAATA
GCATAATTCAAATTTTTGGAATCTTAAATTAGCTACTTGCNGGTTGCTTGTGAAAGGNATATAATG
ATTACATTGTAAACAAATTTAAATATTTATGGATATTTGTGAAAAGCTGCATTATGTTAAATAATATT
ACATGTAAAGCT

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FIGURE 2

MCLSHLENMPLSHSRTQGAQRSSWKLWLFCSIVMLLFLCSFSWLIFIFLQLETAKEPCMAKFGPLPSKW
QMASSEPPCVNKVSDWKLEILQNGLYLIYGQVAPNANYNDVAPFEVRLYKNKDMIQTLTNKSQI QNVGG
TYELHVGD TIDLIFNSEHQVLKNNTYWG IILLANPQFIS

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FIGURE 3

GACATCGGAGGTGGGCTAGCACTGAACTGCTTTTCAAGACGAGGAAGAGGAGGAGAAAGAGAAAGAAG
AGGAAGATGTTGGGCAACATTTATTTAACATGCTCCACAGCCCGGACCCTGGCATCATGCTGCTATTCC
TGCAAATACTGAAGAAGCATGGGATTTAAATATTTTACTTCTAAATAAATGAATTACTCAATCTCCTAT
GACCATCTATACATACTCCACCTTCAAAAAGTACATCAATATTATATCATTAAGGAAATAGTAACCTTC
TCTTCTCCAATATGCATGACATTTTTGGACAATGCAATTGTGGCACTGGCACTTATTTTCAGTGAAGAAA
AACTTTGTGGTTCTATGGCATTATCATTTTGACAAATGCAAGCATCTTCCTTATCAATCAGCTCCTATT
GAACTTACTAGCACTGACTGTGGAATCCTTAAGGGCCCATTACATTTCTGAAGAAGAAAGCTAAG
ATGAAGGACATGCCACTCCGAATTCATGTGCTACTTGGCCTAGCTATCACTACACTAGTACAAGCTGTA
GATAAAAAAGTGATTGTCCACGGTTATGTACGTGTGAAATCAGGCCTTGGTTTACACCCAGATCCATT
TATATGGAAGCATCTACAGTGGATTGTAATGATTTAGGTCTTTAACTTTCCAGCCAGATTGCCAGCT
AACACACAGATTCTTCTCTACAGACTAACAAATATTGCAAAAATTGAATACTCCACAGACTTTCCAGTA
AACCTTACTGGCCTGGATTTATCTCAAAACAATTTATCTTCAGTCACCAATATTAATGTAAAAAGATG
CCTCAGCTCCTTTCTGTGTACCTAGAGGAAAACAACTTACTGAACTGCCTGAAAAATGTCTGTCCGAA
CTGAGCAACTTACAAGAACTCTATATTAATCACAACCTTGCTTTCTACAATTTACCTGGAGCCTTTATT
GGCCTACATAATCTTCTTCGACTTCATCTCAATTCAAATAGATTGCAGATGATCAACAGTAAGTGGTTT
GATGCTCTTCCAATCTAGAGATTCTGATGATTGGGGAAAAATCCAATTATCAGAATCAAAGACATGAAC
TTTAAGCCTCTTATCAATCTTCGCAGCCTGGTTATAGCTGGTATAAACCTCACAGAAATACCAGATAAC
GCCTTGGTTGGACTGGAAGAACTTAGAAAGCATCTCTTTTACGATAACAGGCTTATTAAAGTACCCCAT
GTTGCTCTTCAAAAAGTTGTAAATCTCAAATTTTTGGATCTAAATAAAAAATCCTATTAATAGAATACGA
AGGGGTGATTTTAGCAATATGCTACACTTAAAGAGTTGGGGATAAATAATATGCCTGAGCTGATTTCC
ATCGATAGTCTTGCTGTGGATAACCTGCCAGATTTAAGAAAAATAGAAGCTACTAACAACCCTAGATTG
TCTTACATTACCCCAATGCATTTTTAGACTCCCCAAGCTGGAATCACTCATGTGAACAGCAATGCT
CTCAGTGCCCTGTACCATGGTACCATTGAGTCTCTGCCAAACCTCAAGGAAATCAGCATACACAGTAAC
CCCATCAGGTGTGACTGTGTCATCCGTTGGATGAACATGAACAAAACCAACATTTCGATTTCATGGAGCCA
GATTCATGTTTTGCGTGGACCCACCTGAATTCGAAGGTCAGAATGTTCCGGCAAGTGCATTTTCAGGGAC
ATGATGGAAATTTGTCTCCCTCTTATAGCTCCTGAGAGCTTTCTTCTAATCTAAATGTAGAAGCTGGG
AGCTATGTTTTCTTTTCACTGTAGAGCTACTGCAGAACACAGCCTGAAATCTACTGGATAACACCTTCT
GGTCAAAAACCTTGCCTAATACCCTGACAGACAAGTTCTATGTCCATTCTGAGGGAACTAGATATA
AATGGCGTAACTCCCAAGAAGGGGGTTTATATACTGTATAGCAACTAACCTAGTTGGCGCTGACTTG
AAGTCTGTTATGATCAAAGTGGATGGATCTTTTCCACAAGATAACAATGGCTCTTTGAATATTAAAAA
AGAGATATTAGGCCAATTCAGTTTTGGTGTCTTGGAAAGCAAGTTCTAAAATTCTCAAATCTAGTGTT
AAATGGACAGCCTTTGTCAAGACTGAAAATTCTCATGCTGCGCAAAGTGCTCGAATACCATCTGATGTC
AAGGTATATAATCTTACTCATCTGAATCCATCAACTGAGTATAAAATTTGTATTGATATTCCCACCATC
TATCAGAAAAACAGAAAAAATGTGTAAATGTCACCACCAAGGTTTGACCCCTGATCAAAAAGAGTAT
GAAAAGAATAATACCACAACACTTATGGCCTGTCTTGAGGCCTTCTGGGGATTATTGGTGTGATATGT
CTTATCAGCTGCCTCTCTCCAGAAATGAAGTGTGATGGTGGACACAGCTATGTGAGGAATTACTTACAG
AAACCAACCTTTGCATTAGGTGAGCTTTATCCTCCTGATAAATCTCTGGGAAGCAGGAAAAAGAAAA
AGTACATCACTGAAAGTAAAGCAACTGTTATAGGTTTACCAACAAATATGTCCTAAAAACCACAAGG
AAACCTACTCCAAAAATGAAC

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FIGURE 4

MKDMPLRIHVLLGLAITTLVQAVDKKVDPCRLCTCEIRPWFTPRSIYMEASTVDCNDLGLLTFPARLPA
NTQILLQTNNAKIEYSTDFPVNLTGLDLSQNNLSSVTNINVKKMPQLLSVYLEENKLTPEKCLSE
LSNLQELYINHNLLSTISPGAFIGLHNLLRLHLNSNRLQMINSKWFDALPNLEILMIGENPIIRIKDMN
FKPLINLRSLVIAGINLTEIPDNALVGLENLESISFYDNRLIKVPHVALQKVVLKFLDLNKNPINRIR
RGDFSNNLHLKELGINNMPELISIDSLAVDNLPDLRKIEATNNPRLSYIHPNAFFRLPKLESIMLNSNA
LSALYHGTIESLPNLKEISIHSPNIRCDVCVIRWMNMNKTNI RFMEPDSLFCVDPPEFQGGQNVROVHFRD
MMEICLPLIAPESFPSNLNVEAGSYVSFHCRTAEPPQPEIYWITPSGQKLLPNTLTDKFYVHSEGLDI
NGVTPKEGGLYTCIATNLVGADLKSVMIKVDGSFPQDNNGSLNIKIRDIQANSVLVSWKASSKILKSSV
KWTAFVKTENSAAQSARIPSDVKVYNLTHLNPSTEYKICIDIPTIYQKNRKKCVNVTTKGLHPDQKEY
EKNNTTTLMACLGGLLGIIGVICLISCLSPENMCDGGHSYVRNYLQKPTFALGELYPPLINLWEAGKEK
STSLKVKATVIGLPTNMS

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FIGURE 5

CACAAGCATCTTAATTTGAATCCACAAAGTTTCATGTAATGAAAAGAAATACATAATTTTAATTC AAC
CGAGTGTTTTCCAAGAAGATTGTATTTGCTTAAATTGCTACAGTAATTCAAGAGACAGCCCTGTCTGGA
CACAGAGTTACTGTGGATTTTAAAGAGACTCAGTTAAAGAATTTAGGAATTTCTGATTCATTTAAAGGA
TTTACAAATTCATCAACCCCTGAAAAC TAAAGCAAATTGAACAGGAAAAAAAAAAGAAG
ATGGGTTTTTTAAGTCCAATATATGTTATTTTCTCTTTTGGAGTCAAAGTACATTGCCAATATGAA
ACTTATCAGTGGGATGAAGACTATGACCAAGAGCCAGATGATGATTACCAAACAGGATTTCCATTTCTGT
CAAAATGTAGACTACGGAGTTCCTTTTCATCAGTATACTTTAGGCTGTGTCACTGAATGCTTCTGTCCA
ACTAATTTCCATCATCAATGTACTGTGATAATCGCAAAC TCAAGACTATCCAAATATTTCCGATGCAC
ATTCAGCAACTCTACCTTCAGTTCAATGAAATTGAGGCTGTGACTGCAAATTCATTCATCAATGCAACT
CATCTTAAAGAAATTAACCTCAGCCACAACAAAATTAATCTCAAAAGATTGATTATGGTGTGTTTGCT
AAGCTTCCAAATCTACTACAACTTCATCTAGAGCATAATAATTTAGAAGAATTTCCATTTCTCTCTCT
AAATCTCTGGAAAGACTCCTTCTTGTTACAATGAAATCTCCAAACTGCAGACAAATGCTATGGATGGG
CTAGTAAACTTGACCATGCTTGATCTCTGTTATAATTATCTTCATGATTCTCTGCTAAAAGACAAAATC
TTTGCCAAAATGGAAAACTAATGCAGCTCAACCTCTGCAGTAACAGATTAGAATCAATGCCTCCTGGT
TTGCCTTCTTCACTTATGTATCTGTCTTTAGAAAATAATTCATTTCTTCTATACCCGAAAAATACTTC
GACAAACTTCCAAACTTCATACTCTAAGAATGTCACACAACAAACTACAAGACATCCCATATAATATT
TTTAATCTTCCCAACATTGTAGAACTCAGTGTGGACACAACAAATTGAAGCAAGCATTCTATATTCCA
AGAAATTTGGAACACCTATACCTACAAAATAATGAAATAGAAAAGATGAATCTTACAGTGATGTGTCCT
TCTATTGACCCACTACATTACCACCATTTAACATACATTTCGTGTGGACCAAAATAAACTAAAAGAACCA
ATAAGCTCATACATCTTCTCTGCTTCCCTCATATACACACTATTTATTATGGTGAACAACGAAGCACT
AATGGTCAAACAATACAAC TAAAGACACAAGTTTTTCAGGAGATTTCCAGATGATGATGATGAAAGTGAA
GATCAGATGATCCTGACAATGCTCATGAGAGCCAGAACAAAGAAGGAGCAGAAGGGCACTTTGACCTT
CATTATTATGAAAATCAAGAA TAGCAAGAACTATATAGGTATACACTTACGACTTCACAAAACCTATA
CTTAATATAGTAAATCTAAGTAAACATGTATTACTCAAAGTAATATATTTAGAATTATGTATTAGTATA
AGATCAGAATTGAATTTAAGTTGTTGGTGACATCTGCATCATTTTCATAGGATTAGAACTTACTCAAAAT
AATGTAAATCTTTAAAAATATAAATTAGAATGACAAGTGGGAATCATAAATTAAACGTTAATGGTTTCT
TATGCTCTTTTAAATATAGAAATATCATGTTAAAGAAAAAAAAAAAAA

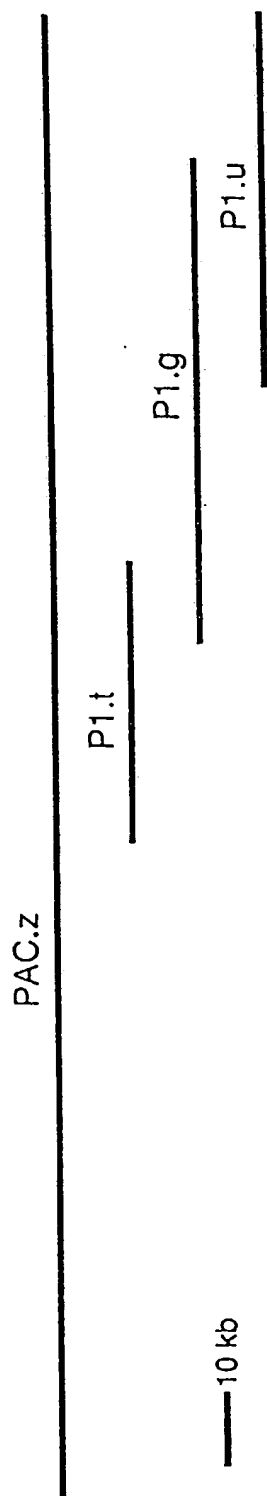
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FIGURE 6

MGFLSPIYVIFFFGVKVCQYETYQWDEDYDQEPDDDYQTGFPRQNVGYGVPFHQYTLGCVSECFP
TNFPSSMYCDNRKLKTI PNIPMHIQQLYLQFNEIEAVTANSFINATHLKEINLSHNKIKSQKIDYGVFA
KLPNLLQLHLEHNNLEEFPLPKSLERLLGYNEISKLTNAMDGLVNLMLDLCYNYLHDSLLKDKI
FAKMEKLMQLNLCSNRLESMPGLPSSLMYLSLENNSSISPEKYFDKLPKLHTLRMSHNKLQDI PYNI
FNLPNIVELSVGHNKLKQAFYIPRNLEHLYLQNNIEKMNLTVMCPSIDPLHYHHLTYIRVDQNKLEP
ISSYIFFCFPHIHTIYYGEQRSTNGQTIQLKTQVFRFPDDDDSESDHDDPDNAHESPEQEGAEGHFDL
HYENQE

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Figure 7



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FIGURE 8

CGGACGCGTGGGCGGACGCGTGGGCCCCGSGCACC GCCCGCCCGGCCCTCCGCCCTCCGCACTCGC
GCCTCCCTCCCTCCGCCGCTCCCGCGCCCTCCTCCCTCCCTCCCGAGCTGTCCCGTTCGCGTC
ATGCCGAGCCTCCCGGCCCGGCCGCCCGCTGCTGCTCCTCGGGCTGCTGCTGCTCGGCTCCCGGCCG
GCCCGCGCGCGCGGCCAGAGCCCCCGTGTGCCCATCCGTTCTGAGAAGGAGCCGCTGCCCGTTCGG
GGAGCGGAGGCTGCACCTTCGGCGGAAGGTCTATGCCTTGGACGAGACGTGGCACCCGGACCTAGGG
CAGCCATTCGGGGTGATGCGCTGCGTGTGCGCCTGCGAGGCGCCTCAGTGGGGTCGCCGTACCAGG
GGCCCTGCGAGGGTCAGCTGCAAGAACATCAAACCAGAGTGCCCAACCCCGGCCCTGTGGGCAGCCGCGC
CAGCTGCCGGGACACTGCTGCCAGACCTGCCCCAGGAGCGCAGCAGTTTCGGAGCGGAGCCGAGCGGC
CTGTCTTCGAGTATCCGCGGGACCCGAGCATCGCAGTTATAGCGACCCGCGGGAGCCAGGCGCTGAG
GAGCGGGCCCGTGGTGACGGCCACACGACTTCGTGGCGCTGCTGACAGGGCCGAGGTGCGAGGCGGTG
GCACGAGCCCGAGTCTCGCTGCTGCGCTCTAGCCTCCGCTTCTCTATCTCCTACAGGCGGCTGGACCGC
CCTACCAGGATCCGCTTCTCAGACTCCAATGGCAGTGTCTGTTTGGACACCTGCAGCCCCACCCAA
GATGGCCTGGTCTGTGGGGTGCGCGGAGCTGCAGATGGCCCTGGAGTGGGAGGCGAGGCGCTG
CTGCATGTGGCACTTGTGACACTCACTCACCTTCAGGGGAGGTCTGGGGGCTCTCATCCGGCACCGG
GCCCTGGCTGCAGAGACCTTCAGTGCCATCCTGACTCTAGAAGGCCCCACAGCAGGGCGTAGGGGGC
ATCACCTGCTCACTCTCAGTGACACAGAGGACTCCTTGCATTTTTGTGCTGCTCTCCGAGGGCTGCTG
GAACCCAGGAGTGGGGGACTAACCAGGTTCCCTTGAGGCTCCAGATTCTACACAGGGGAGCTACTG
CGAGAACTTCAGGCCAATGTCTCAGCCAGGAACAGGCTTTGCTGAGGTGCTGCCAACCTGACAGTC
CAGGAGATGGACTGGCTGGTGTGCTGGGGAGCTGCAGATGGCCCTGGAGTGGGAGGCGAGGCGCTG
CGCATCAGTGGACACATTGCTGCCAGGAAGAGCTGCGACGCTCTGCAAAGTGTCTTTGTGGGGCTGAT
GCCCTGATCCAGTCCAGACGGGTGCTGCCGGCTCAGCCAGCCTCACGCTGCTAGGAAATGGCTCCCTG
ATCTATCAGGTGCAAGTGGTAGGGACAAGCAGTGAGGTGGTGGCCATGACACTGGAGACCAAGCCTCAG
CGGAGGGATCAGCGCACTGTCTGTGCCACATGGCTGGACTCCAGCCAGGAGGACACACGGCCGTGGGT
ATCTGCCCTGGGCTGGGTGCCGAGGGGCTCATATGCTGCTGCAGAAATGAGCTCTTCTGAACGTGGGC
ACCAAGGACTTCCAGACGGAGAGCTTCGGGGGACGCTGGCTGCCCTGCCCTACTGTGGGCATAGCGCC
CGCCATGACACGCTGCCGTGCCCTAGCAGGAGCCCTGGTGTACCCCTGTGAAGAGCCAAGCAGCA
GGGCACGCTGGCTTTCTTGATACCCACTGTCACCTGCACTATGAAGTGCTGCTGGCTGGGCTTGGT
GGCTCAGAAACAAGGCACTGTCACCTGCCACCTCCTTGGGCTCCTGGAACGCCAGGGCTCGGCGGCTG
CTGAAGGGATTCTATGGCTCAGAGGCCAGGGTGTGGTGAAGGACCTGGAGCCGGAAGTGTGCGGCAC
CTGGCAAAGGCATGGCCTCCCTGATGATCACCACCAAGGGTAGCCCCAGAGGGGAGCTCCGAGGGCAG
GTGCACATAGCCAACCAATGTGAGGTTGGCGGACTGCGCCTGGAGGCGCGGGGCGAGGGGTGCGG
GCGCTGGGGGCTCCGGATACAGCCTCTGCTGCGCCGCTGTGGTGCCTGGTCTCCCGCCCTAGCGCCC
GCCAAACCTGGTGGTCTTGGGCGGCCCGAGACCCCAACACATGCTTCTTCGAGGGGCGAGCGCCCC
CACGGGGCTCGCTGGGCGCCCAACTACGACCCGCTCTGCTCACTCTGCACCTGCCAGAGACGAACGGTG
ATCTGTGACCCGGTGGTGTGCCACCGCCAGCTGCCACACCCGGTGCAAGGCTCCCGACCAAGTGTGC
CCTGTTTGCCCTGAGAAACAAGATGTGAGAGACTTGCCAGGGCTGCCAAGGAGCCGGGAGCCAGGAGAG
GGCTGCTATTTTGATGGTGACCGGAGCTGGCGGGCAGCGGGTACGCGGTGGCACCCCGTTGTGCCCCC
TTTGGCTTAATTAAGTGTGCTGTCTGCACCTGCAAGGGGGGCACTGGAGAGGTGCACTGTGAGAAGGTG
CAGTGTCCCGGCTGGCTGTGCCAGCCTGTGCGTGTCAACCCACCGACTGCTGCAAACAGTGTCCA
GTGGGGTCCGGGGGCCACCCCGAGCTGGGGGACCCCATGCAGGCTGATGGGCCCCGGGGCTGCCGTTTT
GCTGGGCAGTGGTTCCAGAGAGTCAAGCTGGCACCCCTCAGTGCCCTTTTGGAGAGATGAGCTGT
ATCACCTGCAGATGTGGGCGAGGGGTGCTCACTGTGAGCGGGATGACTGTTCACTGCCACTGTCTGT
GGCTCGGGGAAGGAGAGTCGATGCTGTCCCGCTGCACGGCCACCGGCGGCCCCAGAGACCAGAAGT
GATCCAGAGCTGGAGAAAGAAGCCGAAGGCTCTTAGGGAGCAGCCAGAGGGCCAAGTGACCAAGAGGAT
GGGGCTGAGCTGGGGAAGGGGTGGCATCGAGGACCTTCTTGCACTTCTCTGTGGGAAGCCAGTGCCT
TTGCTCCTCTGTCTGCTCTACTCCACCCCACTACCTCTGGGAACCAAGCTCCACAAGGGGGAGA
GGCAGCTGGGCGAGACCGAGGTACAGCCACTCCAAGTCTGCCCTGCCACCTCGGCTCTGTCTTG
AAGCCCCACCCCTTTCTCTGTACATAATGTCACTGGCTTGTGGGATTTTAAATTATCTTCACTCA
GCACCAAGGGCCCCGCACTCCACTCTGCTGCCCCGAGCTGAGCAGAGTCATTATTGGAGAGTTTT
GTATTTATTAAACATTCTTTTTTTCAGTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

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FIGURE 9

MPSLPAPPAPLLLLLGLLLGSRPARGAGPEPPVLPIRSEKEPLPVRGAAGCTFGGKVYALDETHPDLG
QPFGVMRCVLCACEAPQWGRTRGPGRVSCKNIECPTPACGQPRQLPGHCCQTCQERSSSERQPSG
LSFEYPRDPEHRSYSDRGEFGAEERARGDGHTDFVALLTGPRSQAVARARVSLRSSLRFSISYRRLDR
PTRIRFSDSNGSVLFEHPAAPTQDGLVCGVWRAVPRLSLRLRAEQLHVALVTLTHPSGEVWGPIRHR
ALAAETFSAILTLEGPPQGVGGITLLTSLDTEDSLHFLLLFRGLLEPRSGGLTQVPLRLQILHQGQLL
RELQANVSAQEPGFAEVLPNLTVQEMDWLVLGELQMALEWAGRPGLRISGHIAARKSCDVLQSVLCGAD
ALIPVQTGAAGSASLTLLGNGSLIYQVQVVGTSSEVVAMTLETKPQRRDQRTVLCHMAGLQPGGHTAVG
ICPGLGARGAHMLLQNELFLNVGTDKDFPDGELRGHVAALPYCGHSARHDTLPVPLAGALVLPVKSQAA
GHAWLSLDTHCHLHYEVLLAGLGGSEQCTVTAHLLGPPGTPGPRRLLKGFYGSEAQGVVKDLEPELLRH
LAKGMASLMITTKGSPRGELRGQVHIANQCEVGGLRLEAAGAEGVRALGAPDTASAAPPVVPGLPALAP
AKPGGPGRPRDPNTCFEFGQQRPHGARWAPNYDPLCSLCTCQRRTVICDPVVCPPPSCPHVQAPDQCC
PVCPEKQDVRLPGLPRSRDPGEGCYFDGDRSWRAAGTRWHPVVPFGLIKCAVCTCKGGTGEVHCEKV
QCPRLACAQPVVRNPTDCKKQCPVGSGAHPQLGDPMQADGPRGCRFAGQWFPESQSWHPSVPPFGEMSC
ITCRGAGVPHCERDDCSLPLSCGSGKESRCCSRCTAHRPPETRTDPELEKEAEGS

FIGURE 10

[illegible]

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FIGURE 11

MRAPGCGRLVLPLLLLAAAAAEGDAKGLKEGETPGNFMEDQWLSSISQYSGKIKHWNRFREVEDDY
IKSWEDNQQGDEALDTTKDPCQKVKCSRHKVCIAQGYQRAMCISRKKLEHRIKQPTVKLHGNKDSICKP
CHMAQLASVCGSDGHTYSSVCKLEQQACLSSKQLAVRCEGPCPCPTEQAATSTADGKPETCTGQDLADL
GDRLRDWFQLLHENSQNGSASSVAGPASGLDKSLGASCKDSIGWMFSKLDTSADLFLDQTELAALND
KYEVCIRPFFNSCDTYKDGRVSTAIEWCFCFWREKPPCLAELEIRIQIEAAKKKPGIFIPSCDEDGYRK
MQCDQSSGDCWRVDQLGLELTGTRTHGSPDCDDIVGFGDFGSGVGWEDEEEKETEEAGEEAEDEGEA
GEADDGGYIW

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FIGURE 12

CTTCTTTGAAAAGGATTATCACCTGATCAGGTTCTCTCTGCATTGCCCCCTTTAGATTGTGAA
ATGTGGCTCAAGGTCTTCACAACTTTCCTTTTCCTTTGCAACAGGTGCTTGCTCGGGGCTGAAGGTGACA
GTGCCATCACAACTGTCCATGGCGTCAGAGGTCAGGCCCTCTACCTACCCGTCCACTATGGCTTCCAC
ACTCCAGCATCAGACATCCAGATCATATGGCTATTTGAGAGACCCACACAATGCCCAAATACTTACTG
GGCTCTGTGAATAAGTCTGTGGTTCCCTGACTTGGAATACCAACACAAGTTCACCATGATGCCACCCAAT
GCATCTCTGCTTATCAACCCACTGCAGTTCCTTGATGAAGGCAATTACATCGTGAAGGTCAACATTGAG
GGAAATGGAATCTATCTGCCAGTCAGAAGATACAAGTCACGGTTGATGATCTGTCACAAAGCCAGTG
GTGCAGATTATCCTCCCTCTGGGGCTGTGGAGTATGTGGGGAACATGACCCTGACATGCCATGTGGAA
GGGGGCACTCGGCTAGCTTACCAATGGCTAAAAATGGGAGACCTGTCCACACCAGCTCCACCTACTCC
TTTTCTCCCCAAAACAATACCCTTCATATTGCTCCAGTAACCAAGGAAGACATTGGGAATTACAGCTGC
CTGGTGAGGAACCCCTGTCAGTGAAATGGAAAGTGATATCATTATGCCCATCATATATTATGGACCTTAT
GGACTTCAAGTGAATTCTGATAAAGGGCTAAAAGTAGGGGAAGTGTACTGTTGACCTTGGAGAGGCC
ATCCTATTTGATTGTTCTGCTGATTCTCATCCCCCAACACCTACTCCTGGATTAGGAGGACTGACAAT
ACTACATATATCATTAAGCATGGGCCTCGCTTAGAAGTTGCATCTGAGAAAGTAGCCAGAAAGACAATG
GACTATGTGTGCTGTGCTTACAACAACATAACCGGCAGGCAAGATGAAACTCATTTACAGTTATCATC
ACTTCCGTAGGACTGGAGAAGCTTGCACAGAAAGGAAAATCATTTGTCACCTTTAGCAAGTATAACTGGA
ATATCACTATTTTTGATTATATCCATGTGTCTTCTCTCCTATGGAAAAATATCAACCCTACAAAGTT
ATAAAACAGAACTAGAAAGGCAGGCCAGAAACAGAATACAGGAAAGCTCAAACATTTTCAGGCCATGAA
GATGCTCTGGATGACTTCGGAATATATGAATTTGTTGCTTTTCCAGATGTTTCTGGTGTTCAGGATT
CCAAGCAGGTCTGTTCCAGCCTCTGATTGTGTATCGGGCAAGATTTGCACAGTACAGTGTATGAAGTT
ATTCAGCACATCCCTGCCCAGCAGCAAGACCATCCAGAG**TGA**ACTTTTCATGGGCTAAACAGTACATTCTG
AGTGAAATCTGAAGAAACATTTTAAGGAAAACAGTGGAAGTATATTAATCTGGAATCAGTGAAGA
AACCAGGACCAACACCTCTTACTCATTATTCTTTACATGCAGAATAGAGGCATTTATGCAAATTGAAC
TGCAGGTTTTTTCAGCATATACACAATGTCTTGTGCAACAGAAAAACATGTTGGGGAAATATTCTCAGT
GGAGAGTCGTTCTCATGCTGACGGGGAGAACGAAAGTGACAGGGGTTTCTCATAAGTTTTGTATGAAA
TATCTCTACAAACCTCAATTAGTTCTACTCTACACTTTCATCATCAACACTGAGACTATCCTGTCT
CACCTACAAATGTGGAACTTTACATTGTTTCGATTTTTCAGCAGACTTTGTTTTATTAAATTTTATTA
GTGTTAAGAAATGCTAAATTTATGTTTCAATTTTATTTCCAAATTTCTATCTTGTTATTTGTACACAAA
GTAATAAGGATGGTTGTCAAAAAACAAAACATGCGCTTCTCTTTTTTTTCAATCACCAGTAGTATTTT
TGAGAAGACTTGTGAACACTTAAGGAAATGACTATTAAAGTCTTATTTTTTATTTTTTTCAAGGAAAGAT
GGATTCAAATAAATTATTCTGTTTTTGCTTTTAAAAA

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FIGURE 13

MWLKVFTTFLSFATGACSGLKVTVPSTVHGVRGQALYLPVHYGFHTPASDIQIIWLFERPHTMPKYLL
GSVNKSVVPDLEYQHKFTMMPPNASLLINPLQFPDEGNYIVKVNIQGNGTLSASQKIQVTVDDPVTKPV
VQIHPPSGAVEYVGNMTLTCHVEGGTRLAYQWLKNGRPVHTSSTYSFSPQNNTLHIAPVTKEDIGNYSC
LVRNPVSEMESDIIMPIIYYGPYGLQVNSDKGLKVGCVFTVDLGEAILEDCSADSHPPNTYSWIRRTDN
TTYIIKHGPRLEVASEKVAQKTM DYVCCAYNNITGRQDETHFTVIITSVGLEKLAQKGKSLSPASITG
ISLFLIISMCLLFLWKKYQPYKVIKQLEGRPETEYRKAQTFSGHEDALDDFGIYEFVAFPDVSGVSR
PSRSVPASDCVSGDLHSTVYEVIQHIPAQQQDHPE

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FIGURE 14

CCCAGGCTCTAGTGCAGGAGGAGAAGGAGGAGGAGCAGGAGGTGGAGATTCCCAGTTAAAAGGCTCCAG
AATCGTGTAACAGGCAGAGAACTGAAGTACTGGGGCCTCCTCCACTGGGTCCGAATCAGTAGGTGACCC
CGCCCCCTGGATTCTGGAAGACCTCACC
ATGGGACGCCCCGACCTCGTGCGGCCAAGACGTGGATGTTCTGCTCTTGCTGGGGGGAGCCTGGGCA
GGACACTCCAGGGCACAGGAGGACAAGGTGCTGGGGGGTTCATGAGTGCCAACCCCATTCGCAGCCTTGG
CAGGCGGCCTTGTTCCAGGGCCAGCAACTACTCTGTGGCGGTGTCCTTGTTAGGTGGCAACTGGGTCCTT
ACAGCTGCCCCTGTAAAAAACCGAAATACACAGTACGCCTGGGAGACCACAGCCTACAGAATAAAGAT
GGCCCAGAGCAAGAAATACCTGTGGTTTCAGTCCATCCACACCCCTGCTACACAGCAGCGATGTGGAG
GACCACAACCATGATCTGATGCTTCTTCAACTGCGTGACCAGGCATCCCTGGGGTCCAAAGTGAAGCCC
ATCAGCCTGGCAGATCATTGCACCCAGCCTGGCCAGAAGTGCACCGTCTCAGGCTGGGGCACTGTCACC
AGTCCCCGAGAGAATTTTCTGACACTCTCAACTGTGCAGAAGTAAAAATCTTTCCCCAGAAGAAGTGT
GAGGATGCTTACCCGGGGCAGATCACAGATGGCATGGTCTGTGCAGGCAGCAGCAAAGGGGGCTGACACG
TGCCAGGGCGATTCTGGAGGCCCCCTGGTGTGTGATGGTGCCTCCAGGGCATCACATCCTGGGGCTCA
GACCCCTGTGGGAGGTCCGACAAACCTGGCGTCTATACCAACATCTGCCGCTACCTGGACTGGATCAAG
AAGATCATAGGCAGCAAGGGCTGAATTCTAGGATAAGCACTAGATCTCCCTTAATAAACTCACAACCTCTC
TGGTTC

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FIGURE 15

MGRPRPRAAKTWMFLLLGGAWAGHSRAQEDKVLGGHECQPHSQPWQAALFQGQQLLCGGVLVGGNWVL
TAAHCKKPKYTVRLGDHSLQNKDGPEQEIPVVQSI PHPCYNSSDVEDHNHDLMLLQLRDQASLGSKVKP
ISLADHCTQPGQKCTVSGWGTVTSPRENF PDTLNCAEVKIFPQKKCEDAYPGQITDGMVCAGSSKGADT
CQGDSGGPLVCDGALQGITSWGSDPCGRSDKPGVYTNICRYLDWIKKIIGSKG

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FIGURE 16

GGCGGCTGCTGAGCTGCCTTGAGGTGCAGTGTGGGGATCCAGAGCC
ATGTCGGACCTGCTACTACTGGGCCTGATTGGGGGCTGACTCTCTTACTGCTGCTGACGCTGCTGGCC
TTTGCCGGGTACTCAGGGCTACTGGCTGGGGTGGAAAGTGAGTGCTGGGTCACCCCCATCCGCAACGTC
ACTGTGGCCTACAAGTTCACATGGGGCTCTATGGTGAGACTGGGCGGCTTTTCACTGAGAGCTGCAGC
ATCTCTCCCAAGCTCCGCTCCATCGCTGTCTACTATGACAACCCCCACATGGTGCCCCCTGATAAGTGC
CGATGTGCCGTGGGCAGCATCCTGAGTGAAGGTGAGGAATCGCCCTCCCCTGAGCTCATCGACCTCTAC
CAGAAATTTGGCTTCAAGGTGTTCTCCTTCCCGGCACCCAGCCATGTGGTGACAGCCACCTTCCCCTAC
ACCACCATTCTGTCCATCTGGCTGGCTACCCGCCGTGTCCATCCTGCCTTGGACACCTACATCAAGGAG
CGGAAGCTGTGTGCCTATCCTCGGCTGGAGATCTACCAGGAAGACCAGATCCATTTTCATGTGCCCACTG
GCACGGCAGGGAGACTTCTATGTGCCTGAGATGAAGGAGACAGAGTGGAATGGCGGGGGCTTGTGGAG
GCCATTGACACCCAGGTGGATGGCACAGGAGCTGACACAATGAGTGACACGAGTTCTGTAAGCTTGGAA
GTGAGCCCTGGCAGCCGGGAGACTTCAGCTGCCACACTGTACCTGGGGCGAGCAGCCGTGGCTGGGAT
GACGGTGACACCCGAGCGAGCACAGCTACAGCGAGTCAGGTGCCAGCGGCTCCTCTTTTGAGGAGCTG
GACTTGGAGGGCGAGGGGCCCTTAGGGGAGTCACGGCTGGACCCTGGGACTGAGCCCTGGGGACTACC
AAGTGGCTCTGGGAGCCCACTGCCCCTGAGAAGGGCAAGGAGTAAACCCATGGCCTGCACCCTCCTGCAG
TGCAGTTGCTGAGGAACTGAGCAGACTCTCCAGCAGACTCTCCAGCCCTCTTCTCCTTCTCTGGGGG
AGGAGGGGTTCCTGAGGGACCTGACTTCCCCTGCTCCAGGCCTTTGCTAAGCCTTCTCCTCACTGCCC
TTTAGGCTCCAGGGCCAGAGGAGCCAGGGACTATTTTCTGCACCAGCCCCAGGGCTGCCGCCCTGT
TGTGTCTTTTTTTCAGACTCACAGTGGAGCTTCCAGGACCCAGAATAAAGCCAATGATTTACTTGTTC
ACCTGGAAAAAAAAAAAAAAAAA

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FIGURE 17

MSDLLLGLIGGLTLLLLLTLLAFAGYSGLLAGVEVSAGSPPIRNVTVAYKFHMGLYGETGRLFTESCS
ISPKLRSIAVYYDNPHMVPPDKCRAVGSI LSEGEESPSPELIDLYQKFGFKVFSFPAPSHVVTATFPY
TTILSIWLATRRVHPALDTYIKERKLCAYPRLEIYQEDQIHMCPLARQGDFFVPEMKETEWKWRGLVE
AIDTQVDGTGADTMSDTSSVSLEVSPGSRETSAA TLSPGASSRGWDDGDTRSEHSYSESGASGSSFEEL
DLEGEGPLGESRLDPGTEPLGTTKWLWEPTAPEKGKE

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FIGURE 18

GCCGCCCCGCCCCGAGACCGGGCCCCGGGGCGCGGGCGGGGATGCGGCGCCCCGGGGCGGGCGATGAC
CGCGGAGCGCACGCCGCGGGCCCCGGCCCTGACCCCGCGCCCGCTGAGCCCCCGCGGAGGTCCG
GACAGGCCGAG
ATGACGCCGAGCCCCCTGTTGCTGCTCCTGCTGCGCGCGCTGCTGCTGGGGGCGCTTCCCACCGGCCGCC
GCCGCCGAGGGCCCCCAAAGATGGCGGACAAGGTGGTCCCACGGCAGGTGGCCCCGCTGGGCCGCACT
GTGCGGCTGCAGTGGCCAGTGGAGGGGACCCGCGCGCTGACCATGTGGACCAAGGATGGCCGCACT
ATCCACAGCGGCTGGAGCCGCTTCCGCGTGCTGCCGCAAGGGCTGAAGGTGAAGCAGGTGGAGCGGGAG
GATGCCGGCGTGACGTGTGAAGGCCACCAACGGCTTCGGCAGCCTGAGCGTCAACTACACCCTCGTC
GTGCTGGATGACATTAGCCCAGGGAAGGAGAGCCTGGGGCCCCGACAGCTCCTCTGGGGGTCAAGAGGAC
CCCCCAGCCAGCAGTGGGCACGACCGCGCTTCACACAGCCCTCCAAGATGAGGCGCCGGGTGATCGCA
CGGCCCCGTGGGTAGCTCCGTGCGGCTCAAGTGCGTGCCAGCGGGCACCCCTCGGCCCGACATCACGTGG
ATGAAGGACGACAGGCCTTGACGCGCCAGAGGCCGCTGAGCCAGGAAGAAGAAGTGGACACTGAGC
GTGAAGAACCTGCGGCGGAGGACAGCGGCAATACACCTGCCGCGTGTCGAACCGCGGGGCGCCATC
AACGCCACCTACAAGGTGGATGTGATCCAGCGGACCCGTTCCAAGCCGCTGCTCACAGGCACGACCCCC
GTGAACACGACGGTGGACTTCGGGGGGACACGTCCTTCCAGTGCAAGGTGCGCAGCGACGTGAAGCCG
GTGATCCAGTGGCTGAAGCGCGTGGAGTACGGCGCCGAGGGCCGCCACAACCTCCACCATCGATGTGGGC
GGCCAGAAGTTTGTGGTGCTGCCACGGGTGACGTGTGGTGCGGGCCCCGACGGCTCCTACCTCAATAAG
CTGCTCATCACCCGTGCCCCGCCAGGACGATGCGGGCATGTACATCTGCCTTGGCGCCAACACCTAGGGC
TACAGCTTCCGACGCGCTTCTCACCCTGCTGCCAGACCCAAAACCGCCAGGGCCACCTGTGGCCCTCC
TCGTCTCCGCCACTAGCCTGCCGTGGCCGCTGGTTCATCGGCATCCAGCGGGCGCTGTCTTCATCCTG
GGCACCTGCTCCTGTGGCTTTGCCAGGCCCCAGAAGAAGCCGTGCACCCCCGCGCCTGCCCTCCCCCTG
CCTGGGCACCGCCCCGCGGGGACGGCCCGGACCGCAGCGGAGACAAGGACCTTCCCTCGTTGGCCGCC
CTCAGCGCTGGCCCTGGTGTGGGGCTGTGTGAGGAGCATGGGTCTCCGGCAGCCCCCAGCACTTACTG
GGCCCAGGCCAGTTGCTGGCCCTAAGTTGTACCCCAAACCTCTACACAGACATCCACACACACACAC
ACACACTCTCACACACACTCACACGTGGAGGGCAAGTCCACCAGCACATCCACTATCAGTGCT**AGACG**
GCACCTATCTGCAGTGGGCACGGGGGGGCGGCCAGACAGGCAGACTGGGAGGATGGAGGACGGAGCT
GCAGACGAAGGCAGGGGACCCATGGCGAGGAGGAATGGCCAGCACCCAGGCAGTCTGTGTGTGAGGCA
TAGCCCCCTGGACACACACACAGACACACACACTACCTGGATGCATGTATGCACACACATGCGCGCAC
ACGTGCTCCCTGAAGGCACACGTACGCACACGCACATGCACAGATATGCCGCTGGGCACACAGATAAG
CTGCCCAAATGCACGCACACGCACAGAGACATGCCAGAACATACAGGACATGCTGCCTGAACATACAC
ACGCACACCCATGGCGAGATGTGCTGCCTGGACACACACACACACAGGATATGCTGTCTGGACGCACA
CACGTGCAGATATGGTATCCGGACACACAGTGCACAGATATGCTGCCTGGACACACAGATAATGCTGC
CTTGACACACACATGCACGGATATTGCCTGGACACACACACACACACACGCGTGCACAGATATGCTGTC
TGGACACGCACACACATGCAGATATGCTGCCTGGACACACACTCCAGACACACGTGCACAGGCGCAGA
TATGCTGCCTGGACACACGAGATATGCTGTCTAGTCACACACACACGACAGATGCTGTCCGGACACA
CACACGCATGCACAGATATGCTGTCCGGACACACACACGACGACAGATATGCTGCCTGGACACACAC
AGATAATGCTGCCTCAACACTCACACAGTGCAGATATTGCCTGGACACACACATGTGCACAGATATGC
TGTCTGGACATGCACACAGTGCAGATATGCTGTCCGGATACACACGACGCACACATGCAGATATGCT
GCCTGGGCACACACTTCCGGACACACATGCACACACAGGTGCAGATATGCTGCCTGGACACACACAG
ATAATGCTGCCTCAACACTCACACAGTGCAGATATTGCCTGGACACACACATGTGCACAGATATGCTG
TCTGGACATGCACACAGTGCAGATATGCTGTCCGGATACACACGACGCACACATGCAGATATGCTGC
CTGGGCACACACTTCCGGACACACATGCACACACAGGTGCAGATATGCTGCCTGGACACACGCACTG
ACGTGCTTTTGGGAGGGTGTGCCGTGAAGCCTGCAGTACGTGTGCCGTGAGGCTCATAGTTGATGAGGG
ACTTCCCTGCTCCACCGTCACTCCCCAACTCTGCCCGCTCTGTCCCCGCTCAGTCCCCGCTCCA
TCCCCGCTCTGTCCCTGGCCTTGGCGGCTATTTTGGCACCTGCCTTGGGTGCCAGGAGTCCCCCTA
CTGCTGTGGGCTGGGGTGGGGGACAGCAGCCCCAAGCCTGAGAGGCTGGAGCCCATGGCTAGTGGCT
CATCCCCAGTGCATTCTCCCCCTGACACAGAGAAGGGGCTTGGTATTTATATTTAAGAAATGAAGATA
ATATTAATAATGATGAAGGAAGACTGGGTGACGGGACTGTGGTCTCTCCTGGGGCCCCGGACCCGCC
TGGTCTTTTCAGCCATGCTGATGACCACCCCCGTCCAGGCCAGACACCACCCCCACCCACTGTCCGT
GTGGCCCCAGATCTCTGTAATTTTATGTAGAGTTTGAGCTGAAGCCCCGTATATTTAATTTATTTTGT
AAACACAAA

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FIGURE 19

MTSPLLLLLLPPLLGAFFPAAAARGPPKMADKVVPRQVARLGRTVRLQCPVEGDPPPLTMWTKDGRT
IHSGWSRFRVLPQGLKVQVEREDAGVYVCKATNGFGSLSVNYTLVVLDISP GKESLGPDSSSGQED
PASQQWARPRFTQPSKMRRRV IARPVGSSVRLKCVASGHPRPDITWMKDDQALTRPEAAEPRKKKWTLS
LKNLRPEDSGKYTCRVSNRAGAINATYKVDVIQRTSRKPVLGTGHPVNTTVDFGGTTSFQCKVRSDVKP
VIQWLKRVEYGAEGRHNSTIDVGGQKFVVLPTGDVWSRPDGSYLNKLLITRARQDDAGMYICLGANTMG
YSFRSAFLT VLPDPKPPGPPVASSSSATSLPWPVVIGIPAGAVFILGTL LLWLCQAQKKPCTPAPAPPL
PGHRPPGTARDRSGDKDLPSLAALSAGPGVGLCEEHGSAPAQHLLGPGFPVAGPKLYPKLYTDIHTH
THSHTHSHVEGKVHQHIHYQC

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FIGURE 20

GCCTAGCCAGGCCAAGA

ATGCAATTGCCCCGGTGGTGGGAGCTGGGAGACCCCTGTGCTTGGACGGGACAGGGTCGGGGGACACGC
AGGATGAGCCCCGCGACCACTGGCACATTCTTGCTGACAGTGTACAGTATTTTCTCCAAGGTACACTCC
GATCGGAATGTATACCCATCAGCAGGTGTCCTCTTTGTTTCATGTTTTGGAAAGAGAATATTTTAAGGGG
GAATTTCCACCTTACCCAAAACCTGGCGAGATTAGTAATGATCCCATAAACATTTAATACAAATTTAATG
GGTTACCCAGACCGACCTGGATGGCTTCGATATATCCAAAGGACACCATATAGTGATGGAGTCCTATAT
GGGTCCCCAACAGCTGAAAATGTGGGGAAGCCAACAATCATTGAGATAACTGCCTACAACAGGCGCACC
TTTGAGACTGCAAGGCATAATTTGATAATTAATATAATGTCTGCAGAAGACTTCCCGTTGCCATATCAA
GCAGAAATCTTCATTAAGAATATGAATGTAGAAGAAATGTTGGCCAGTGAGGTTCTTGGAGACTTTCTT
GGCGCAGTGAAAAATGTGTGGCAGCCAGAGCGCCTGAACGCCATAAACATCACATCGGCCCTAGACAGG
GGTGGCAGGGTGCCACTTCCCATTAAATGACCTGAAGGAGGGCGTTTATGTCATGGTTGGTGCAGATGTC
CCGTTTTCTTCTTGTGTTACGAGAAGTTGAAAATCCACAGAATCAATTGAGATGTAGTCAAGAAATGGAG
CCTGTAATAACATGTGATAAAAAATTTCTGACTCAATTTTACATTGACTGGTGCAAAATTTTATTGGTT
GATAAAACAAAGCAAGTGTCCACCTATCAGGAAGTGATTCGTGGAGAGGGGATTTTACCTGATGGTGGGA
GAATACAAACCCCTTCTGATTCTTTGAAAAGCAGAGACTATTACACGGATTTCTTAATTACACTGGCT
GTGCCCTCGGCAGTGGCACTGGTCCTTTTTCTAATACTTGCTTATATCATGTGCTGCCGACGGGAAGGC
GTGGAAAAGAGAAACATGCAAACACCAGACATCCAAGTGGTCCATCACAGTGTATTTCAGAAATCTACC
AAGGAGCTTCGAGACATGTCCAAGAATAGAGAGATAGCATGGCCCTGTCAACGCTTCTGTGTTCCAC
CCTGTGACTGGGGAATCATACTCTTTTACACACAGACAATATGATAGCACAAACATGCCATTGATG
CAAACGCAGCAGAACTTGCCACATCAGACTCAGATTCCCCAACAGCAGACTACAGGTAAATGGTATCCC
TGAAGAAAGAAAACCTGACTGAAGCAATGAATTTATAATCAGACAATATAGCAGTTACATCACATTTCTT
TTCTCTTCCAATAATGCATGAGCTTTTCTGGCATATGTTATGCATGTTGGCAGTATTAAGTGATACCA
AATAATACAACATACTTTTCAATTTTACTAATGTATTTTTTGTACTTAAAGCATTTTGGACAATTTGTA
AAACATTGATGACTTTATATTTGTTACAATAAAAGTTGATCTTTAAATAAATATTATTAATGAAGCCT
AAAAAAAAAA

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FIGURE 21

MQLPRWWELGDPCAWTGQGRGTRRMSPATTGTFLTVYSIFSKVHSDRNVYPSAGVLFVHVLEREYFKG
EFPPYPKPGEISNDPITFNTNLMGYPDPRPGWLRYIQRTPYSDGVLYGSPTAENVGKPTIIETAYNRRT
FETARHNLIIINIMSAEDFPLPYQAEFFIKNMNVEEMLASEVLGDFLGAVKNVWQPERLNAINITSALDR
GGRVPLPINDLKEGVYVMVGADVPFSSCLREVENPONQLRCSQEMEPVITCDKKFRTQFYIDWCKISLV
DKTKQVSTYQEVIRGEGILPDGGEYKPPSDSLKSRDYYTDFLITLAVPSAVALVFLILAYIMCCRREG
VEKRRMQTPDIQLVHHS AIQKSTKELRDMSKNREIAWPLSTLPVFHPVTGEIIPPLHTDNYDSTNMPLM
QTQQNLPHQTQIPQQTTGKWYP

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FIGURE 22

CGGACGCGTGGGCCGCCACCTCCGGAACAAGCC
ATGGTGGCGGCGACGGTGGCAGCGGCGTGGCTGCTCCTGTGGGCTGCGGCCTGCGCGCAGCAGGAGCAG
GACTTCTACGACTTCAAGGCGGTCAACATCCGGGGCAAAC TGGTGTGCTGGAGAAGTACCGCGGATCG
GTGTCCCTGGTGGTGAATGTGGCCAGCGAGTGGGCTTACAGACCAGCACTACCGAGCCCTGCAGCAG
CTGCAGCGAGACCTGGGCCCCACCACCTTTAACGTGCTCGCCTTCCCCTGCAACCAGTTTGGCCAACAG
GAGCCTGACAGCAACAAGGAGATTGAGAGCTTTGCCCGCCGCACCTACAGTGTCTCATTCCCCATGTTT
AGCAAGATTGCAGTCACCGGTACTGGTGGCCATCCTGCCTTCAAGTACCTGGCCCAGACTTCTGGGAAG
GAGCCCACCTGGAACCTTCTGGAAGTACCTAGTAGCCCCAGATGGAAAGGTGGTAGGGGCTTGGGACCCA
ACTGTGTCAGTGGAGGAGGTCAGACCCCAGATCACAGCGCTCGTGAGGAAGCTCATCCTACTGAAGCGA
GAAGACTTATAACCACCGCGTCTCCTCCTCCACCACCTCATCCCGCCACCTGTGTGGGGCTGACCAAT
GCAAACTCAAAATGGTGTCTCAAAGGGAGAGACCCACTGACTCTCCTTCCTTTACTCTTATGCCATTGGT
CCCATCATTCTTGTGGGGGAAAAATTCTAGTATTTTGATTATTTGAATCTTACAGCAACAAATAGGAAC
TCCTGGCCAATGAGAGCTCTTGACCAGTGAATCACCAGCCGATACGAACGTCTTGCCAACAAAAATGTG
TGGCAAATAGAAGTATATCAAGCAATAATCTCCACCCAAGGCTTCTGTAACTGGGACCAATGATTAC
CTCATAGGGCTGTTGTGAGGATTAGGATGAAATACCTGTGAAAGTGCCTAGGCAGTGCCAGCCAAATAG
GAGGCATTCAATGAACATTTTTTGCATATAAACCAAAAAATAACTTGTTATCAATAAAAACTGCATCC
AACATGAATTTCCAGCCGATGATAATCCAGGCCAAAGGTTTAGTTGTTGTTATTTCTCTGTATTATTT
TCTTCATTACAAAAGAAATGCAAGTTCATTGTAACAATCCAAACAATACCTCACGATATAAAATAAAAA
TGAAAGTATCCTCCTCAAAAA

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FIGURE 23

MVAATVAAAWLLLWAAACAQQEQDFYDFKAVNIRGKLVSLVKYRGSVSLVVNVASECGFTDQHYRALQQ
LQRDLGPHHFENVLAFPCNQFGQQEPDSNKEIESFARRTYSVSFPMFSKIAVTGTGAHPAFKYLAQTSGK
EPTWNFWKYL VAPDGKVVGAWDPTVSVEEVRPQITALVRKLILLKREDL

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FIGURE 24

AAACTTGACGCC

ATGAAGATCCCGGTCTTCCTGCCGTGGTGCTCCTCTCCCTCCTGGTGCTCCACTCTGCCCAGGGAGCC
ACCCTGGGTGGTCTTGAGGAAGAAAGCACCATTGAGAATTATGCGTCACGACCCGAGGCCTTTAACACC
CCGTTCTCGAACATCGACAAATTGCGATCTGCGTTTAAGGCTGATGAGTTCCTGAACTGGCACGCCCTC
TTTGAGTCTATCAAAGGAAACTTCCTTTCCTCAACTGGGATGCCTTTCCTAAGCTGAAAGGACTGAGG
AGCGCAACTCCTGATGCCCAGTGACCATGACCTCCACTGGAAGAGGGGGCTAGCGTGAGCGCTGATTCT
CAACCTACCATAACTCTTCTGCCTCAGGAACCCAATAAAACATTTTCATCCAA

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FIGURE 25

MKIPVLPVAVLLSLLVLHSAQGATLGGPEEESTIENYASRPEAFNTPFLNIDKLRSAFKADEFNLNWHAL
FESIKRKL PFLNWDAFPKLKGLRSATPDAQ

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FIGURE 26

CAACAGAAGCCAAGAAGGAAGCCGTCTATCTTGTGGCGATC
ATGTATAAGCTGGCCTCCTGCTGTTTGCTTTTCACAGGATTCTTAAATCCTCTCTTATCTCTTCCTCTC
CTTGACTCCAGGGAAATATCCTTTCAACTCTCAGCACCTCATGAAGACGCGCGCTTAATCCGGAGGAG
CTAGAAAGAGCTTCCCTTCTACAGATATTGCCAGAGATGCTGGGTGCAGAAAGAGGGGATATTCTCAGG
AAAGCAGACTCAAGTACCAACATTTTAAACCAAGAGGAAATTTGAGAAAGTTTCAGGATTTCTCTGGA
CAAGATCCTAACATTTTACTGAGTCATCTTTGGCCAGAATCTGGAAACCATAACAAGAAACGTGAGACT
CCTGATTGCTTCTGGAAATACTGTGTCT**GAA**GTGAAATAAGCATCTGTTAGTCAGCTCAGAAACACCCA
TCTTAGAATATGAAAAATAACACAATGCTTGATTGAAAACAGTGTGGAGAAAACTAGGCAAACCTACA
CCCTGTTCAATTGTTACCTGGAAAATAAATCCTCTATGTTTGCACAAAAAAAAAAAAAAAAA

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FIGURE 27

..MYKLASCCLLFTGFLNPLLSLPLDSREISFQLSAPHEDARLTPEELERASLLQILPEMLGAERGDILR
KADSSTNIFNPRGNLRKFQDFSGQDPNILLSHLLARIWKPYKKRETPDCFWKYCV

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FIGURE 28

GCCCCAGGGAGCAGTGGGTGGTTATAACTCAGGCCCGGTGCCAGAGCCCAGGAGGAGGCAGTGGCCAG
GAAGGCACAGGCCTGAGAAGTCTGCGGCTGAGCTGGGAGCAAATCCCCACCCCCTACCTGGGGGACAG
GGCAAGTGAGACCTGGTGAGGGTGGCTCAGCAGGCAGGGAAGGAGAGGTGTCTGTGCGTCTGCACCCA
CATCTTTCTCTGTCCCTCCTTGCCCTGTCTGGAGGCTGCTAGACTCCTATCTTCTGAATTCTATAGTG
CCTGGGTCTCAGCGCAGTGCCGATGGTGGCCCGTCCTTGTGGTTCTCTCTACCTGGGGAAATAAGGTG
CAGCGGCC
ATGGCTACAGCAAGACCCCCTGGATGTGGGTGCTCTGTGCTCTGATCACAGCCTTGCTTCTGGGGGTC
ACAGAGCATGTTCTCGCCAACAATGATGTTTCCTGTGACCACCCCTCTAACACCGTGCCCTCTGGGAGC
AACCAGGACCTGGGAGCTGGGGCCGGGAAGACGCCCCGTGGATGACAGCAGCAGCCGCATCATCAAT
GGATCCGACTGCGATATGCACACCCAGCCGTGGCAGGCCGCGCTGTTGCTAAGGCCCAACCAGCTCTAC
TGCGGGGCGGTGTTGGTGCATCCACAGTGGCTGCTACGGCCGCCCACTGCAGGAAGAAAGTTTTCAGA
GTCCGTCTCGGCCACTACTCCCTGTCAACAGTTTATGAATCTGGGCAGCAGATGTTCCAGGGGGTCAAA
TCCATCCCCACCCCTGGCTACTCCCACCTGGCCACTCTAACGACCTCATGCTCATCAAACCTGAACAGA
AGAATTCGTCCCCTAAAGATGTCAGACCCATCAACGTCTCCTCTCATTGTCCCTCTGCTGGGACAAAG
TGCTTGGTGTCTGGCTGGGGGACAACCAAGAGCCCCCAAGTGCACCTCCCTAAGGTCCTCCAGTGCTTG
AATATCAGCGTGCTAAGTCAGAAAAGGTGCGAGGATGCTTACCCGAGACAGATAGATGACACCATGTTT
TGCGCCGGTGACAAAGCAGGTAGAGACTCCTGCCAGGGTGATTCTGGGGGGCCTGTGGTCTGCAATGGC
TCCCTGCAGGGAAGTCGTGCTCTGGGAGATTACCTTGTGCCCGGCCCAACAGACCGGGTGTCTACACG
AACCTCTGCAAGTTCACCAAGTGGATCCAGGAAACCATCCAGGCCAACTCCTGAGTCATCCAGGACTC
AGCACACCGGCATCCCCACCTGCTGCAGGGACAGCCCTGACACTCCTTTCAGACCTCATTCCTTCCCA
GAGATGTTGAGAATGTTTCATCTCTCCAGCCCCTGACCCCATGTCTCCTGGACTCAGGGTCTGCTTCCCC
CACATTGGGCTGACCGTGTCTCTCTAGTTGAACCCCTGGGAACAATTCCAAAACCTGTCCAGGGCGGGG
TTGCGTCTCAATCTCCCTGGGGCACTTTCATCCTCAAGCTCAGGGCCCATCCCTTCTCTGCAGCTCTGA
CCCAATTTAGTCCCAGAAATAAACTGAGAAGTGGAACCAAAAAA

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FIGURE 29

MATARPPWMWVLCALITALLGVTEHVLANNVSCDHPSNTVPSGSNQDLGAGAGEDARSDDSSSRIIN
GSDCDMHTQPWQAALLLRPNQLYCGAVLVHPQWLLTAAHCRKKVFRVRLGHYSLSPVYESGQQMFQGVK
SIPHPGYSHPGHSNDLMLIKLNRRIRPTKDVRPINVSSHCP SAGTKCLVSGWGTTKSPQVHF PKVLQCL
NISVLSQKRCE DAYPRQIDDTMFCAGDKAGR DSCQGD SGGPVVCNGSLQGLVSWGDYPCARPNRPGVYT
NLCKFTKWIQETIQANS